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THE INCISOR TEETH OF ALBINO RATS AND GUINEA PIGS IN VITAMIN A DEFICIENCY AND REPAIR *

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In 1925 [1] we described very briefly two outstanding effects of vitamin A deficiency upon the incisor teeth of white rats — atrophy and metaplasia of the enamel organ and atrophy of odontoblasts accompanied by atypical formations of dentine. Since then we have studied the teeth of vitamin A deficient guinea pigs and added considerably to our understanding of the deficiency changes by the study of the sequences of repair in white rats during recovery induced by the addition of butter fat to the diet. We emphasize the value of following recovery changes in all experimental studies of the consequences of vitamin deficiencies. In vitamin A deficiency, as in vitamin C deficiency (Wolbach and Howe [2]), cells of mesenchymal origin undergo changes in morphology, making identification inaccurate or impossible. In recovery we have found that such cells, osteoblasts and odontoblasts, resume function before morphological restoration is complete. The presence or absence of a deposit of matrix around cells by way of a recovery response and the character of the matrix if deposited, as well as the restored morphology of the cells, are decisive factors in identification of cells which concern us in studies of teeth in deficiencies.

Few histological studies have been made upon teeth in vitamin A deficiency. May Mellanby has written extensively and wisely upon the importance of vitamin A for the normal development of teeth. These papers fortunately are summarized in an excellent review [3]

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and present definite proof that defective or absent dentine and enamel formations are consequences of vitamin A deficiency. In her studies of human and experimental animal material, undecalcified ground sections were employed so that cell responses and histological sequences could not be followed. Marshall,[4] without giving histological details, attributed the formation of "pulp stones" (calcification in the pulp) in white rats to vitamin A deficiency. Coolidge[5] mentioned and illustrated irregularities of the dentine and the formation of osteoid masses free in the pulp or attached to the dentine in one white rat "of several hundred." The diet of this rat was not given and he gave no histological details, but as the illustrations are those of conditions constant in vitamin A deficiency, we believe that intentionally or inadvertently this rat's diet was deficient in vitamin A. More recently, Shibata[6] reported as consequences of vitamin A deficiency in white rats "abnormal formation of the enamel, dentin and cementum; degenerative changes such as atrophy and abnormality in enamel organ dental pulp and root membrane tissues; retardation of the eruption of the incisors; and the changes in the chemical compositions such as the decrease of phosphorous and calcium, increase of water magnesium, etc." Shibata refers to earlier papers of his but does not give the references so that we could not consult them for the histological descriptions which the above-mentioned paper lacks. It seems probable that many of the details we shall describe were seen by Shibata.

Our endeavor, as part of a program to achieve specific histopathological characterizations of the avitaminoses, has been to follow the sequences due to A deficiency in the teeth for the purpose of ascertaining the initial or direct (specific) effects, as contrasted with indirect or non-specific effects such as may be the consequences of secondary general disturbances in metabolism. Accordingly sections of decalcified skulls were used. Fixation was either in Zenker's liquid or 10 per cent formalin. Five per cent nitric acid in 70 per cent alcohol was used for decalcifying. Celloidin sections stained with hematoxylin and eosin were routinely used, but various special stains for connective tissue and reticulin were employed when indicated.

The diet used for the rats was that described by us in 1925. Whether or not the absence of vitamins C, D, and E was of importance we cannot say. We adhered to it because (1) scorbutic effects cannot be produced in rats; (2) vitamin D is not necessary to prevent

rickets in rats with properly balanced inorganic salts in their diets when kept under proper hygienic conditions; (3) we know of no effects of vitamin E deficiency outside of the testes in the male and the products of conception in the female; and (4) of greatest importance, the addition of vitamin A alone sufficed to bring about histological recovery.

The diet used for the guinea pigs was that described by us in 1928.[7] We varied the amount of orange juice in an attempt to find an amount which would prevent scorbutus and yet allow an A deficiency. Twenty cc. of orange juice daily is sufficient to prevent histological effects of vitamin A deficiency in any organ in guinea pigs of 300 to 500 gm. weight. Eight cc. daily does not prevent the epithelial metaplasia of vitamin A deficiency. Two cc. daily is sufficient to prevent scorbutic lesions in bones. The majority of our guinea pigs received 4 to 8 cc. of orange juice daily. This we emphasize because certain findings in the teeth of guinea pigs which were reported by Höjer [8] are apparently duplicated in guinea pigs on vitamin A deficient diet plus 4 to 8 cc. of orange juice daily.

Our studies were made from frontal plane sections through the skull at three or more levels each, including the upper incisor teeth. Owing to the curvature of the incisor tooth the frontal plane sections do not give true cross-sections, except in the midportion of the tooth. Allowance must be made for this in viewing the illustrations because the sections through anterior and posterior portions of the tooth cut the labial and lingual walls obliquely. The lower incisors were studied in sections at the level of the first molars in rats with different degrees of the deficiency. In guinea pigs and in rats during recovery the lower jaws were sectioned at three levels, one through the first molars, and two posterior to that.

The following tables serve to indicate the scope of our material.

The Incisor Teeth of Rats

The gross changes have not been accurately followed by us. Growth of the teeth seems to be commensurate with the growth of the skull, which shares in the marked retardation of the skeleton as a whole. The teeth lose the normal orange pigmentation and acquire a chalky-white appearance, due we believe to loss of the enamel which is the pigmented part, and to change in composition of the

TABLE I

Vitamin A Deficient Rats Used for Tooth Studies

No. of rat	Duration of deficient diet		Metaplasia all organs
	days		
227	61	Died	+
224	63	"	+++
207	77	"	++
209	82	"	+
205	89	"	++
206	89	"	++
220	91	"	+++
222	97	"	+++
203	98	"	++
201	100	Killed	++
202	101	"	+++
238	114	"	++
101	115	"	+
31	149	Died	++++
35	149	Killed	++
105	160	Died	+
107	167	"	+
106	171	"	+
103	180	"	++++
100	181	Killed	+

TABLE II

Vitamin A Deficient Rats in Recovery Used for Tooth Studies

No. of rat	Deficiency period	Repair period	Metaplasia of epitheliums	Weights in grams			
				Initial	Maximum	End of deficiency	Recovery
	days	*days*					
212	70	9	++	95	128	105	?
239	103	5	++	63	123	99	?
425	118	10	+	141	162	147	144
232	137	6	+	114	152	120	?
235	138	5	+	104	140	126	85
397	138	12	+	84	136	119	135
393	143	18	+	85	128	109	125
394	146	22	+	81	119	80	105
413	150	13	++	135	136	104	107+
399	151	15	+	86	140	123	142
400	151	20	++	81	129	107	129+
401	161	22	++	81	114	87	117
412	170	14	++	128	137	126	169
414	170	19	+++	143	146	112	160
50	185	43	++	?	?	?	?
417	187	7	+++	133	133	102	120

In the last column of weights + means that the recorded weight was that of several days previous to killing.

TABLE III

Vitamin A Deficient Guinea Pigs Used for Tooth Studies

No. of guinea pig	Duration of deficiency	Metaplasia all organs	Orange juice daily
	days		*cc.*
2	66	+	2
28	67	++	8–2*
29	67	++	8–2*
24	71	++	20–2*
38	101	?	16–4
23	105	+++	20–4–0**
42	105	+	16–8
39	110	+++	16–2
6	112	?	20–8
142	128	+++	4
45	144	?	20–8
15	153	++	20–4
46	158	?	20–8
143	160	++	4
72	161	++	16–2
8	163	++	20–8
30	287	0	20
41	299	++	20–5

* These three guinea pigs, after the orange juice was reduced to 4 cc. daily, received none for 2 weeks and then received 2 cc. daily for the final 2 weeks of life.

** This guinea pig received no orange juice for the final 2 weeks of life.

dentine. Also, the teeth are more brittle than normal. Striking histological changes were present in all the teeth of the rats listed in Table I so that a pronounced effect resulting in change of cross-sectional shape is apparent in from 60 to 70 days, possibly earlier. At all levels we find the dentine on the labial side of the tooth disproportionately thick, compared to the lingual, lateral and mesial sides. Figures 1 and 3 are typical of A deficiency. In advanced deficiency (Figs. 3 and 4) there may be gaps in the wall of dentine on either side, but always near the basal, open end of the tooth. An early effect of the deficiency is atrophy and cessation of function of the odontoblasts. The odontoblasts on the labial side (adjacent to the enamel organ) are affected last and this accounts for the final cross-sectional shape of the tooth. We have not determined the rate of growth of the incisors in vitamin A deficiency. In the normal albino rat (Addison and Appleton [9]) the rate of growth of the upper incisors is given as 2.2 mm. per week, of the lowers, 2.8 mm. per week.

Addison and Appleton also give the following lengths in millimeters of the incisors at different ages.

Age	Upper incisors	Lower incisors
23 days	12.8	18.1
41 "	15.0	21.7
10 weeks	18.3	25.5
15 "	20.3	26.4
5 months	23.3	29.4
8 "	23.7	29.9
10 "	26.2	31.3

If in vitamin A deficiency the *rate* of growth of the incisor teeth in *relation* to the growth of the skeleton as a whole is approximately that of the normal animal, it is probable, and we believe certain, that the entire tooth is replaced through growth during dietary periods covered by our observation. This must be kept in mind in studying sections taken at different lengths from the formative end of the tooth.

The enamel organ which extends for the entire length of the incisor tooth on its labial surface (elsewhere only about 1 mm. from the basal formative end) early undergoes atrophy and metaplasia (Fig. 2) and consists finally of atrophic remnants of the epithelial papillae and squamous cells replacing the ameloblasts and stratum intermedium (see Fig. 5 for contrast). The anterior part of the enamel organ is first affected but finally the whole length is involved, including the basal formative end (Rat 31, Table I). At the basal end there is continuous renewal of the enamel organ and in many rats with marked metaplasia in many organs practically normal structures were found here, though with distorted relationships, owing to pressure deformity of the tooth because of defective or absent dentine formation. According to Addison and Appleton the ameloblasts travel forward as the incisor grows and it is a fact of interest that for a brief period these cells, as well as the rest of the enamel organ, may retain normal structural appearances. The effect of the deficiency is most apparent after the ameloblasts have reached the region where they are normally most active in enamel formation. Toward the occlusal end, though still within the area of functional enamel organ, the metaplasia may become complete and as a result the enamel organ may be replaced by an epidermis-like structure with superficial keratinized cells. In middle and posterior parts we have not seen this degree of metaplasia. In the atrophy of the enamel organ (Fig. 5) the epithelial papillae are affected first. These structures shrink in size because of atrophy of cells and the surrounding connective tissues become less vascular. The ameloblasts diminish in size, become

coarsely granular and finally disappear. Cell outlines are lost and the ameloblast layer becomes represented by a layer of granular material and pyknotic nuclei, and then disappears. Cells presumably of the stratum intermedium persist as flat cells, usually two rows deep, until in very late stages in the anterior half of the tooth these develop numerous layers of flat cells, the most superficial of which occasionally become keratinized. Very rarely, in contrast to its occurrence in guinea pigs, after complete loss of ameloblasts globular formations of calcified material develop in the connective tissue adjacent to atrophied papillae (Fig. 6). This calcification following atrophy of the structure whose function it is to receive and segregate calcium salts from the blood stream, invites speculations regarding the possibilities of selective permeabilities of the capillaries supplying the epithelial papillae.

The sequences determined by study of rats in varying degrees of the deficiency are supported by the sequences of repair during recovery induced by feeding butter fat. The first evidence of recovery in the enamel organ is the plumping of cells of the epithelial papillae. This is accompanied by engorgement of capillaries and a less dense appearance of the connective tissue immediately adjacent to the papillae. The earliest response was in Rat 417 (Table II) after 7 days of treatment. Subsequently, and in a very few days, the surface cells, presumably of stratum intermedium origin, become cuboidal in shape and continue to assume more and more the appearance of ameloblasts (Rats 397 and 399, Table II, for example). In rats responding favorably to treatment, as shown by rapid gain of weight, the enamel organ is practically normal in appearance on the 19th day (Rat 414, Table II).

The details of changes in the pulp and odontoblasts are more difficult to follow than those in the enamel organ. Simultaneously with changes in the enamel organ occurs atrophy of the odontoblasts but, as we have said above, the odontoblasts on the labial side (adjacent to the enamel organ) retain their morphology and function for long periods. This accounts for the extraordinary thickness of the dentine on the labial side, as contrasted with the remainder of the tooth. It is naturally a pertinent suggestion that the enamel organ, even though undergoing atrophy and separated by a wall of dentine and enamel, still exerts an influence upon the maintenance of the odontoblasts comparable to that in the fetus and at the basal formative end.

Conversely, one may argue that all of the changes in the odontoblasts are secondary to atrophy of the enamel organ and withdrawal of its influence. At the basal formative end the odontoblasts undergo early atrophy, except on the convex or labial surface.

The atrophy of odontoblasts, with the exception of those opposite the enamel organ, proceeds rapidly and is complete usually when general histological changes in many organs are well established, and therefore in as short a period as 60 to 70 days. All cells resembling odontoblasts disappear and the internal surface of the dentine becomes bounded by cells that are indistinguishable from other connective tissue cells of the pulp until extreme thinning of the dentine has resulted (Fig. 7).

During the process of atrophy, odontoblasts, and rarely capillaries that supply them, become incorporated into the dentine because some cells remain functional for a longer time than others and by continuing to form it surround non-functioning cells with dentine. This detail is most strikingly seen when the odontoblasts on the labial side finally begin to atrophy (Fig. 8).

In rare instances, not accounted for by us, cells resembling osteoblasts in size and shape were present on the inner surface of the dentine and had deposited a thin interrupted layer of bone (secondary dentine?).

In the depths of the pulp small areas of osteoid tissue were frequently found in rats with fully developed deficiency. Going over the whole series of rats we worked out the sequences of pulp ossification as follows: first, minute spherical globules of hyaline matrix appear between the pulp cells and simultaneously narrow zones of similar material form about occasional capillaries; these deposits increase and incorporate pulp cells which assume the morphology of osteoblasts, and finally cells in contact with the periphery acquire the morphology of osteoblasts and presumably function as such.

In consequence of the atrophy of odontoblasts, as the tooth continues to grow, the dentine near the formative end becomes very thin, often sharply folded or pleated, or entirely absent (Figs. 3, 9, 10 and 13). Associated with these late consequences of the deficiency are aggregations of cells of two types, each responsible for the formation of masses of dentine (denticles) continuous with or separated from the tooth wall. In every instance where our sections passed through or near to the basal formative end, when these cell aggrega-

tions were present we found strikingly defective dentine formation associated with atrophic odontoblasts and much atrophied ameloblasts in Hertwig's sheath (the enamel organ at the basal formative end).

In some instances columnar cells were enclosed within folds of dentine and the relationships were such as to indicate positively that these cells were incorporated ameloblasts. The morphology indicated also that these cells were ameloblasts because they were cylindrical and radially arranged in contrast to the shrunken odontoblasts on the opposite side of the dentine. In regions of complete absence of the dentine at or close to the formative end there were frequently, in the gaps, gland-like clusters of cells that we are also forced to interpret as of enamel organ origin. Such gland-like formations also lie under the thin abnormal dentine (without canaliculi) at considerable distances from the basal end (Figs. 11, 12 and 15). One type of cell aggregate then we regard as of enamel organ origin and due to the fact that cells (presumably ameloblasts because, as we shall show below, they do not survive in repair) become incorporated by the pleating of a very thin and atypical dentine at the formative end.

The other type of cell aggregate is proliferative in type and consists of cells derived from the pulp that forms in regions of extremely thin or totally absent dentine. These cells are short, plump, basic staining, and resemble transitional forms between mesenchymal cells and osteoblasts. Their presence is accompanied by deposition of atypical dentine (osteodentine) upon the tooth wall (Figs. 3, 9, 10 and 11), although in very severe deficiency they form no matrix at all. These cell aggregates we regard as reparative in origin, comparable to callus formation in bone, and indicative that even in extreme vitamin A deficiency proliferative responses on the part of connective tissues may be energetic. We have further proof of this in the repair of wounds made for experimental purposes. All of our decisions regarding origins of cells were made after careful study of the histology of repair during recovery induced by substituting butter fat for lard in the diet.

An outline of the sequences observed in recovery is therefore indicated. As we have described above, the restoration of the enamel organ takes place first at the basal formative end. Very promptly the odontoblasts in juxtaposition to the enamel organ at this end recover and by the 7th day (Rat 417, Table II) in the region of Hert-

wig's sheath they have assumed normal morphology and function, as shown by the deposition of predentine with canaliculi. The predentine calcifies rapidly. Folds in pleated dentine fill in rapidly. Excess atypical dentine, so-called secondary dentine or osteodentine, the product of the proliferative pulp cell aggregates and denticles of ameloblast inclusion origin grow in size by the deposition of dentine upon them (Figs. 16, 17 and 18). The ultimate fate of these "denticles" we have not followed, but it seems reasonably certain that they continue to increase in size while advancing forward as the tooth grows.

The reappearance of columnar odontoblasts is represented in all stages by our series of repair experiment rats. It takes place first at the basal formative end and works forward, first upon the labial side of the tooth, presumably in response to the recovery of the enamel organ. Later it extends throughout the tooth. Predentine formation is active before the odontoblasts have acquired columnar shapes. Complete morphological recovery was almost achieved in 19 days in Rat 414, which showed a very satisfactory increase of weight in response to butter fat in the diet. There is little to record about the sequences of odontoblast recovery. The changes observed at different periods of recovery were quite like those to be seen in the formation of osteoblasts in granulation tissue in the repair of bone. Connective tissue cells in contact with the dentine become plumper and more basic in staining reaction. For a brief period they are polyhedral in shape and processed like young osteoblasts. Soon they become aligned more or less parallelly with their long diameters oblique or perpendicular to the dentine, but before there is any regularity in arrangement or uniformity in shape, predentine is deposited upon the old dentine. The sequences may be arranged in four stages: (1) increase of size, or at least of cytoplasm concentrated about the nucleus; (2) assumption of osteoblast-like morphology; (3) evidences of function and polarity, as evidenced by directional deposit of predentine; and finally, (4) further growth in size and acquisition of the normal shape.

Various stages of odontoblast recovery may be seen in one tooth because, as stated above, the recovery begins first opposite the enamel organ, *i. e.* at the basal end and labial surface. We have thus had ample opportunity in our recovery series to feel secure in com-

paring the recovery or reappearance of odontoblasts to the sequences seen in bone repair from fibroblast to osteoblast. Our observations lead us to regard, from the morphological viewpoint, the odontoblast as a polarized osteoblast.

The recovery sequences permit no doubt regarding the nature of the proliferative cell aggregates. They are of pulp connective tissue origin. The other types of cell aggregate in gland-like formation we have already concluded were of enamel organ origin. Again the morphological evidence is supplied by the sequences seen in recovery. The first change in recovery is an increase in size of the cells, some of which undergo mitosis (Fig. 12). The pulp cells in contact with these gland-like formations *respond, as do those in contact with the dentine,* and proceed to deposit predentine. We thus get the picture of epithelial cells in the pulp surrounded by dentine, in turn surrounded by odontoblasts. This is shown in low power at A and B in Figure 13 and at higher power in Figure 14. The enclosed epithelial cells undergo atrophy and disappear (Fig. 19). The last stage of degeneration is like that of the ameloblasts in the deficiency. The cells break up into coarse granules and the residue becomes calcified; pyknotic nuclei can be seen in denticles of this origin for some time after calcification of the cell débris (Fig. 18).

Excessive local formation of atypical dentine (denticles) is produced in vitamin A deficiency by focal proliferative responses of pulp cells. Such deposits are insignificant, but increase in the recovery period commensurate with rate and extent of recovery. Another type of excess dentine formation or denticle is initiated by the presence of cells of the enamel organ that become incorporated by folds of dentine or through total gaps in the dentine. In repair these cells stimulate the formation of odontoblasts from the adjacent pulp and hence denticle formation. We thus have brought new experimental evidence to support the prevailing opinion (Orban [10]) that cells of Hertwig's sheath may be carried forward into the pulp and stimulate the formation of odontoblasts from pulp cells, and consequently the formation of denticles. Fridrichovsky [11] described in human teeth and in rats' teeth (diet not stated) folds of dentine such as were of common occurrence in our series. These he regarded as due to developmental irregularities of Hertwig's sheath. He expressed the opinion that such folds could carry enamel organ cells

forward, and thus accounted for one source of denticle formation. Naturally he did not consider deficiency disease as a possible cause of the mechanism.

The Incisor Teeth of Guinea Pigs

In general the effect of vitamin A deficiency upon the incisor teeth of guinea pigs is similar to that in rats. There are some quantitative differences and some apparent contradictions appear to our interpretation of events from the teeth of rats. The outstanding difference is the rapidity and severity of the enamel organ changes. The apparent contradiction is exhibited in the prolonged persistence of the morphology and function of odontoblasts after marked degrees of enamel organ atrophy.

The durations of the deficient diet in the experiments with guinea pigs were on the whole shorter than those with rats, inasmuch as at the beginning the majority of the guinea pigs received sufficient orange juice to afford very considerable amounts of vitamin A. Twenty cc. of orange juice daily is completely protective against histological vitamin A effects in all tissues, except occasionally in the respiratory mucosa in the nares. Sixteen cc. daily is nearly protective. Therefore, the durations given in Table III are not those of completely deficient diets. For example, Guinea pig 38 received 16 cc. of orange juice daily for 56 days, 8 cc. daily for 21 days and 4 cc. daily for 24 days. Guinea pig 15 received 20 cc. for 6 days, 8 cc. for 90 days and 4 cc. for 57 days. Guinea pig 42 received 16 cc. for 55 days, 8 cc. for 50 days; Guinea pig 39 received 16 cc. for 63 days, 4 cc. for 7 days and 2 cc. for 40 days. Guinea pig 6 received 20 cc. for 70 days and 8 cc. for 39 days. Guinea pig 45 received 20 cc. for 69 days and 8 cc. for 75 days. Guinea pig 46 received 20 cc. for 28 days and 8 cc. for 130 days. Guinea pig 8 received 20 cc. for 52 days and 8 cc. for 11 days. Guinea pig 41 received 20 cc. for 209 days, 10 cc. for 15 days and 5 cc. for 75 days.

Gross evidence of scorbutus was absent in each of the guinea pigs in Table III. Histological evidence was found in the costochondral junctions of Guinea pig 23 which received no orange juice during the last two weeks of the experiment. These changes were so slight as to suggest that the retardation of growth secondary to the A deficiency was a factor, inasmuch as there was the typical athreptic type of

costochondral junction. In Guinea pigs 24, 28 and 29 there were microscopic evidences of repaired scorbutus in the teeth, such as we described in 1926.[2] The scorbutic factor, as possibly responsible for the changes we found in our vitamin A deficient guinea pigs, may be dismissed because of the fact that all of the changes we are describing were found in guinea pigs that had never received less than 8 cc. of orange juice daily and that exhibited no gross or microscopic evidences of scorbutus. This we emphasize because Höjer [8] has described and illustrated changes involving odontoblasts and dentine, with the production of osteoid or bony formations in the pulp and upon the dentine in latent or partial scorbutus. As our own work with scorbutus has been upon animals completely deprived of vitamin C we have as yet no basis for criticizing Höjer's conclusions. Such premises as we now have incline us to believe that in partial vitamin C deficiency Höjer's results would be duplicated. Both vitamin A and vitamin C deficiencies affect odontoblasts, though by different routes, but the effect upon dentine formation may be the same.

The Enamel Organ

The first demonstrable effect of the deficiency upon the incisor teeth is in the enamel organ. As in the case of the rat the external layer atrophies first. The ameloblasts become smaller and shorter. In rapidly progressing deficiency they finally undergo granular degeneration and disintegrate. Usually low cuboidal cells persist for long periods and we are not able to say positively whether they are atrophied ameloblasts or cells of the stratum intermedium, though we believe they are derived from the latter, inasmuch as often these cells have horizontal diameters nearly double the vertical, and it is improbable that atrophied cells should undergo such a change. Eventually the enamel organ completely disappears, unlike what occurs in rats. Before the atrophy is complete globules of calcified matrix appear in the outer layer of the enamel organ between the inconspicuous epithelial papillae and within the epithelium itself. These globules increase in size and number, extending into the adjacent connective periodontal membrane. Similar calcified deposits form above the atrophic remains of the enamel epithelium and enclose epithelial cells (Figs. 20 and 21). When large, these calcified deposits take on the appearance of bone (Figs. 22 and 23), because

of the incorporated cells. However, the cells do not have the appearance of osteoblasts. They are without processes and in the largest plaques are surrounded by clear spaces reminiscent of cartilage rather than bone. Neither do these plaques become surrounded by osteoblasts. They resemble cementum rather than other examples of pathological bone formations, such as we are familiar with. They are often bounded on the deep side by cuboidal epithelial cells that laterally are continuous with atrophic cells of the enamel organ. The plaques increase by accretion. Globules of matrix continue to be deposited from the adjacent connective tissue.

The Odontoblasts

Atrophy of the odontoblasts occurs late. In no guinea pigs of our series was there complete cessation of dentine formation; always there were on the labial side a normal-appearing layer of odontoblasts and evidences of functional activity; on mesial and lateral sides only partial atrophy was found and hence the cross-sectional shape peculiar to the rat's incisors was not seen in the guinea pig. As in the case with rats the atrophy of odontoblasts appeared first on the lingual side of the tooth and never before advanced atrophy of the enamel organ had occurred. A very striking difference from occurrences in rat teeth is that coincident with the atrophy of odontoblasts, spicules of a modified dentine form (Fig. 24). This material, at first uncalcified and similar to predentine in all respects except that it contains no canaliculi, becomes finally calcified and continues to grow by peripheral deposit. In long-continued deficiencies, when these dentine-like deposits extend deeply into the pulp, they become surrounded by osteoblasts which they incorporate as they grow; further increase in size is like that of growth of osteoid tissue (Fig. 25).

The earliest formation of these amorphous dentine processes we found in regions of most marked atrophy of the odontoblasts and always upon the lingual side of the tooth. The initial stage we found to be a deposit of predentine-like matrix between atrophied odontoblasts that had lost their columnar form. It was evident that functional activity continued, but polarity of the odontoblasts was lost. In early formations but few cells are found enclosed in matrix. The outgrowths for a period are surrounded by other atrophic cells of the

odontoblast layer which retain their polarity. Finally, polarity is lost and the cells concerned in further growth are indistinguishable from osteoblasts.

The Pulp: The pulp remains practically unchanged. The only ossification we found in our series took origin in the manner described above as a continuation of growth of dentinoid processes.

The Cementum: As a rule in well established A deficiency there are local increases in thickness of the cementum, for the narrow, deeply calcified border surrounding the dentine is to be regarded as cementum. In fact the only cementum resembling bone we have seen in connection with the incisors of guinea pigs has been in A deficiency animals. The formation of cementum of appreciable thickness and enclosing cells is always found first upon the lingual side, but may extend on lateral and mesial sides forward to the enamel organ region as an irregular layer (Fig. 24). Frequently sharply circumscribed cementum formations were found, perhaps warranting application of the term cementicle (Fig. 26).

Discussion

Three facts are of assistance in interpreting vitamin A deficiency effects upon incisor teeth of rodents: (1) the enamel organ moves forward with the growth of the tooth so that the ameloblasts maintain throughout the same relative position to calcified structures; (2) the dentine increases in thickness progressively toward the anterior (occlusal) end, indicating activity of the odontoblasts extending nearly to the end of the tooth; and (3) the occlusal end of the pulp chamber is being constantly filled in by bony material (osteodentine), indicating a normal tendency on the part of pulp cells to produce osteoid matrix and cessation of a polar deposition of matrix on the part of the odontoblasts.

Fact 1 is of assistance in explaining why the earliest and maximum atrophy of the enamel organ is found at some distance from the formative end as a requirement of time for the deficiency to take effect. Fact 2, as dentine is not resorbed in consequence of the deficiency, explains the greater thickness of dentine for long periods in the deficiency toward the occlusal end. Fact 3 warrants the assumption that the deposit of osteodentine following atrophy of odontoblasts is an adaptation or premature stimulation of a normal sequence.

The enamel organ changes we have described in rats and guinea pigs are primary consequences of vitamin A deficiency common to many epithelial organs. The more rapidly produced and pronounced changes in guinea pigs, as compared with rats, are in conformity with the changes in other organs in these two animals (Wolbach and Howe [1]).

We prefer to interpret some effects upon odontoblasts as secondary to enamel organ atrophy, assuming that the latter, in teeth that are continuously growing, exerts an influence upon the former, as suggested by the early sequences in tooth formation and throughout life at the basal formative end of rodents' incisors. This interpretation is not supported by the facts that odontoblasts persist after disappearance of the enamel-forming organ in all stationary permanent teeth of animals, and that in incisor teeth of rodents the enamel-forming organ is continued forward only upon the labial side. Nevertheless, our observations show that enamel organ atrophy precedes atrophy and depolarization of odontoblasts. The fact that in rats odontoblasts remain morphologically and functionally active on the labial side, in apposition to the enamel organ, long after complete disappearance upon other surfaces, and that in this situation their eventual atrophy only follows enamel organ atrophy, demands the above interpretation. The finer sequences attending atrophy of the odontoblasts on the labial side, which we have carefully followed, here and there an odontoblast ceasing to deposit matrix upon its external pole, with continued deposition centrifugally, support strongly the argument that even though the odontoblast may be individually physiologically independent of the enamel organ, architecturally it is dependent. We have come to regard the odontoblast as a polarized osteoblast and the enamel organ as the polarizing agent. In guinea pigs the same changes occur, though in less evident degree. The persistence of normal odontoblasts upon the labial side is more pronounced than in rats. Odontoblast atrophy is less striking, yet illustrates more conspicuously in the sequences we have described the effects of the depolarization of odontoblasts, as shown by the extensions into the pulp of the osteodental processes (denticles) built up by these depolarized cells.

Late vitamin A deficiency effects in bones, as described by Pappenheimer,[12] and completely confirmed by us in unpublished studies, include complete cessation of growth. It may, therefore, be argued

that effects similar to those in bone should be expected in odontoblastic activities and that both are of the same nature. This we may admit without affecting the rôle of the enamel epithelium in the determination of the order of events. More direct attack based upon experimentally produced lesions of the enamel organ is needed to settle these problems.

The formation of bone in rats in the pulp proceeds, we have learned, as does the ossification in membranous bone formation, through direct change in character of the intercellular matrix, preceded by finely granular calcium deposition. Calcification in and adjacent to the atrophied enamel organ, followed by ossification, in guinea pigs may be regarded as a phenomenon having similar pathogenesis. In both instances the cells directly responsible for the elaboration of specific calcified structures, dentine and enamel, are partly or completely inactive, a fact that invites speculations along the lines of a continued extracapillary delivery of calcium and phosphorous compounds to the tissues, and therefore to questions of differentiated permeability of capillary endothelium. The high mineral content of enamel and its rapid growth rate present problems concerning concentration that make such speculations attractive.

We have presented two origins of osteodentine excrescences, or denticles, one occurring in both rats and guinea pigs by the depolarization of odontoblasts, and in the rat accompanied by a proliferative reparative response of pulp cells; the other, only in rats, induced by inclusions of ameloblasts in folds of recently formed dentine, late in the deficiency, close to the formative end. These solutions as to origin could not have been achieved without having for study many stages of repair.

We have observed no changes in the exceedingly thin calcified zone surrounding the dentine in rats which by some morphologists is regarded as cementum. In guinea pigs definite cementum overgrowths (cementicles) were frequently seen on the lingual side of the teeth in consequence of the deficiency, but we can offer no explanation except the suggestion that they formed in response to structural weakness of the bony socket as a part of the general athreptic skeletal effect of late vitamin A deficiency.

The application of our observations to human teeth obviously must be restricted to the formative period and particularly to teeth of the second dentition. Our inference, based upon studies of experi-

mental scorbutus,[2] and rickets (unpublished), is that vitamin A deficiency is the most important because of its effect upon the enamel organ and because recognition of this deficiency is always late and will continue to be so, unless other criteria than ocular effects are looked for by pediatricians. Defective enamel formation, ossification of the pulp, denticle and cementicle formation are all possible consequences of vitamin A deficiency in humans. Studies of unerupted teeth from infants in vitamin A deficiency, obtained post-mortem, are being made by Boyle,[13] and he has recorded observations that indicate we may safely apply some of our observations to the human.

Summary and Conclusions

1. The initial effect of vitamin A deficiency upon incisor teeth of rats and guinea pigs is upon the enamel organ. The ameloblasts respond earliest by atrophy, then the remainder of the organ atrophies; finally metaplasia and calcification, and, in the guinea pig, ossification occur.

2. Atrophy and depolarization of odontoblasts follow enamel organ changes. The odontoblasts survive longest on the side (labial) where in apposition to the enamel organ and in long-continued experiments gross deformities in the incisors of rats resulted from absent or deficient dentine formation.

3. Two types of denticle formation are described, one built up by depolarized odontoblasts, the other by inclusions of ameloblasts by the folding of imperfectly formed dentine at the formative end of the tooth.

4. Defective enamel formation and other poorly understood conditions in teeth, such as denticles, pulp bone and cementicles, may reasonably be regarded in the human as vitamin A deficiency possibilities.

5. Our observations indicate that in the incisor teeth of rodents the odontoblasts throughout life are influenced by the enamel organ.

6. As in other morphological problems concerning vitamin deficiencies, study of the sequences of repair was essential. We emphasize the importance of two types of control material, the normal and progressive stages in repair.

7. Our observations indicate that vitamin A deficiency is the most important of the known vitamin deficiencies in its effect upon tooth formation.

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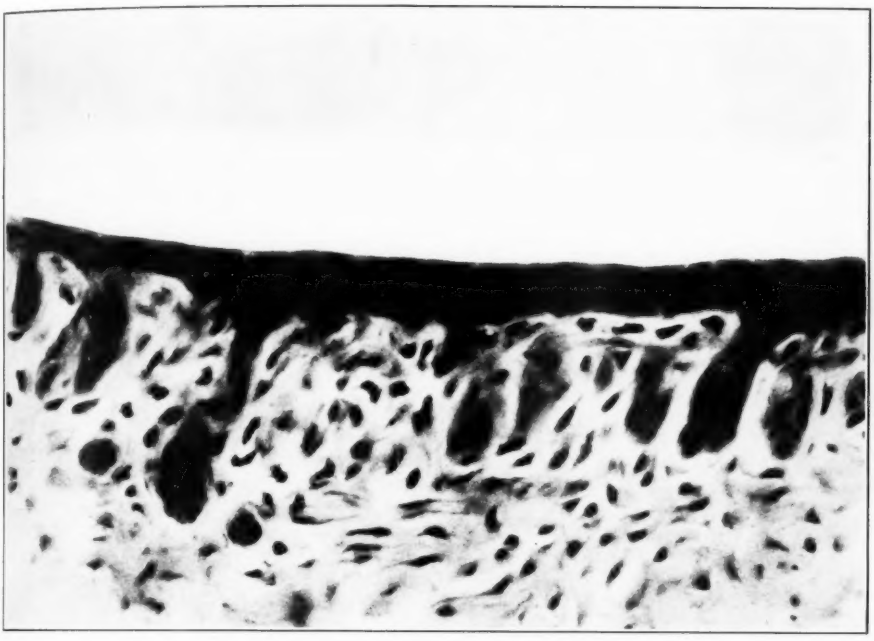
DESCRIPTION OF PLATES

PLATE 38

FIG. 1. Rat 201. Upper incisor, typical of A deficiency in the rat. 101 days on vitamin A deficient diet. Atrophy and metaplasia of the enamel organ. Odontoblasts still present on labial side where the dentine is abnormally thick. Note thinness and folding of dentine on both sides of tooth where there are also many large areas of cellular proliferation derived from pulp cells and possibly from odontoblasts. Osteoid formation present in pulp. × 46.2.

FIG. 2. Rat 201. High power of enamel organ. Same preparation as Fig. 1. 101 days on vitamin A deficient diet. Note great atrophy of the epithelial papillae, absence of ameloblasts and the several layers of flattened cells upon the surface. × 588.



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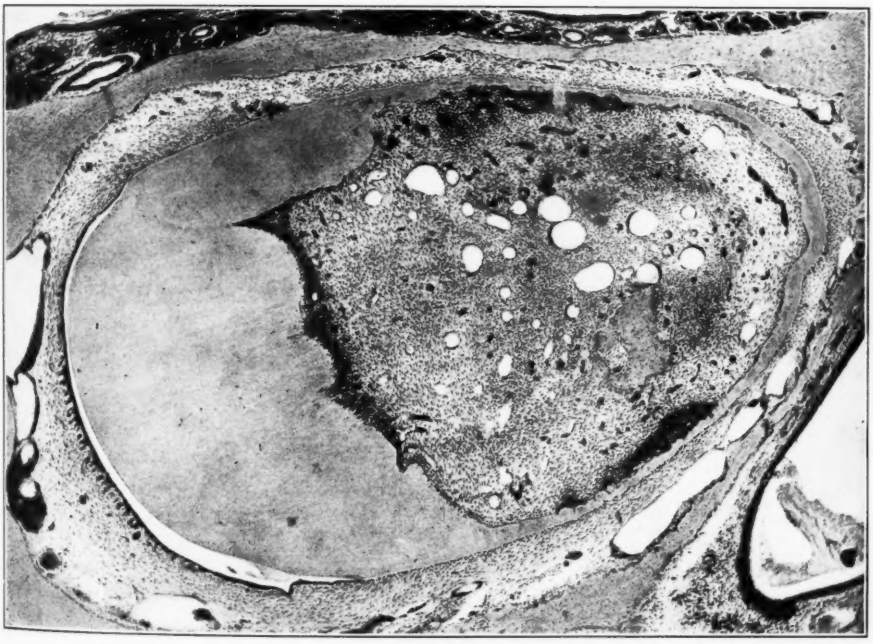


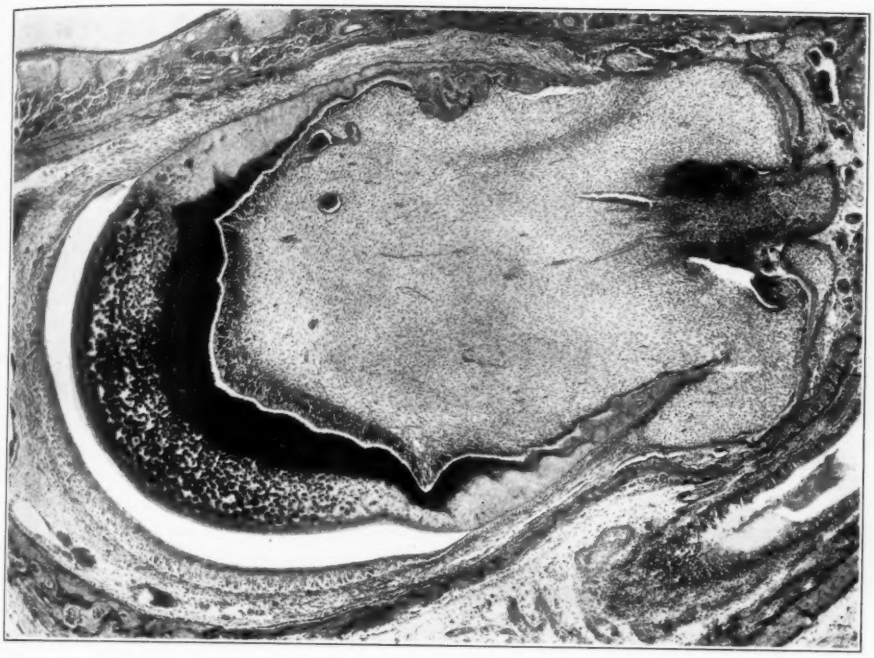
1

Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

PLATE 39

FIG. 3. Rat 205. Upper incisor. 89 days on vitamin A deficient diet. Atrophy and metaplasia of enamel organ. Columnar odontoblasts still present on labial side. On mesial and lateral sides are gaps in the dentine which is elsewhere thin and much folded. Note the large areas of proliferative response composed of cells resembling osteoblasts. × 46.2.

FIG. 4. Rat 412. Upper incisor. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. There is restoration of the enamel organ and heavy calcification of the dentine. The layer of recovering odontoblasts and newly deposited dentine (predentine) shows clearly on both sides, also folding and gaps of the dentine with gross distortion of the shape of the tooth. On the mesial side there are several groups of ameloblasts incorporated in folds of the dentine and further embedded by dentine formed during repair. × 46.2.



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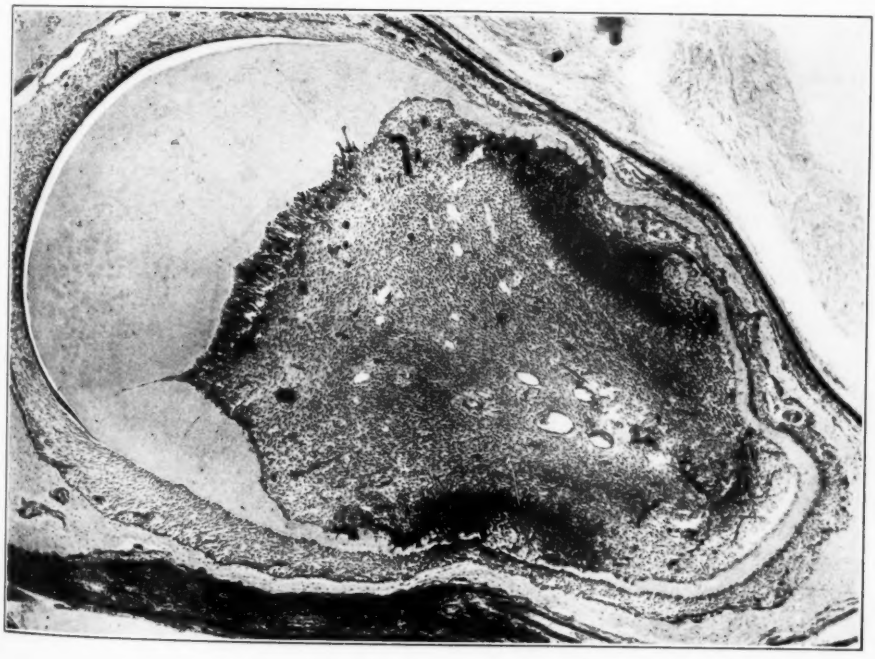

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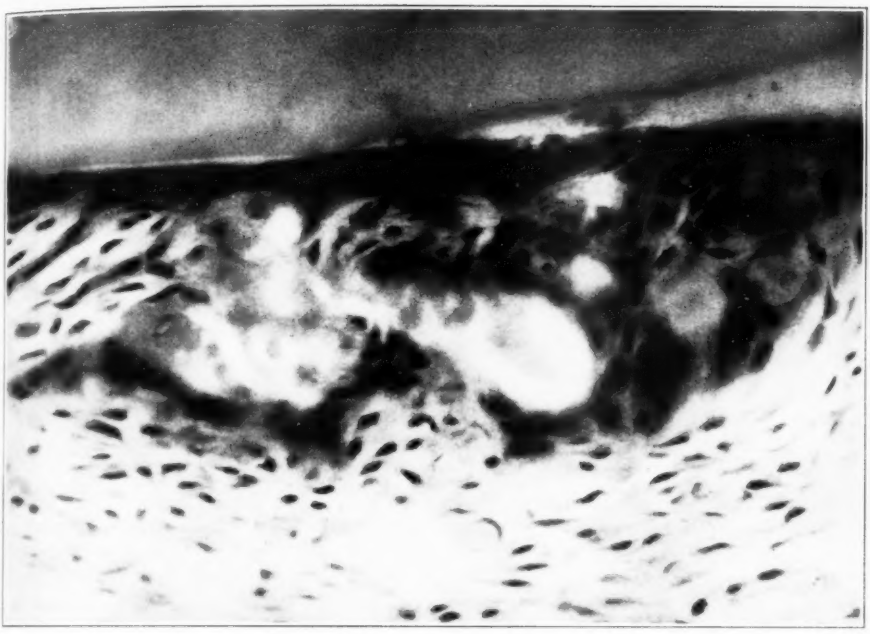
Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

PLATE 40

FIG. 5. The enamel organ of rat fed upon the control diet in which butter fat replaced the lard of the deficient diet. × 588.

FIG. 6. Rat 235. Upper incisor. 138 days on vitamin A deficient diet followed by 5 days with addition of butter fat. Shows extreme atrophy of enamel organ with globular deposits of calcified material in and adjacent to the atrophic remains of the epithelial papillae. × 588.



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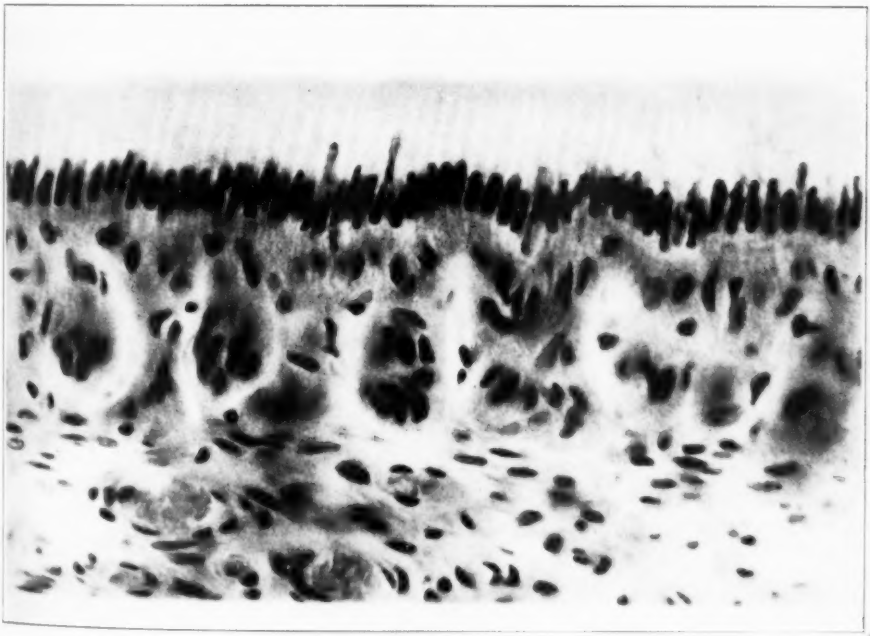

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Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

PLATE 41

FIG. 7. Rat 412. Upper incisor at junction of mesial and labial sides. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. The preparation illustrates the differences in behavior of the odontoblasts of the labial side from those on mesial and lateral sides. The odontoblasts of the first show deposition of dentine under influence of restored diet. On the mesial wall the dentine is bounded by connective tissue-like cells, though early repair is shown in places by traces of dentine deposit between cells, some of which are arranged perpendicularly to the dentine. Note also nuclei of cells incorporated in the dentine. × 147.

FIG. 8. Rat 235. Upper incisor. 138 days on A deficient diet followed by 5 days with addition of butter fat. Shows typical shape of vitamin A deficiency rat incisor. Complete atrophy with metaplasia of the enamel organ. Complete atrophy of odontoblasts except on the labial side where the atrophy is nearly complete. Continued function of some cells has resulted in the incorporation of others by the most recently deposited dentine. × 46.2.

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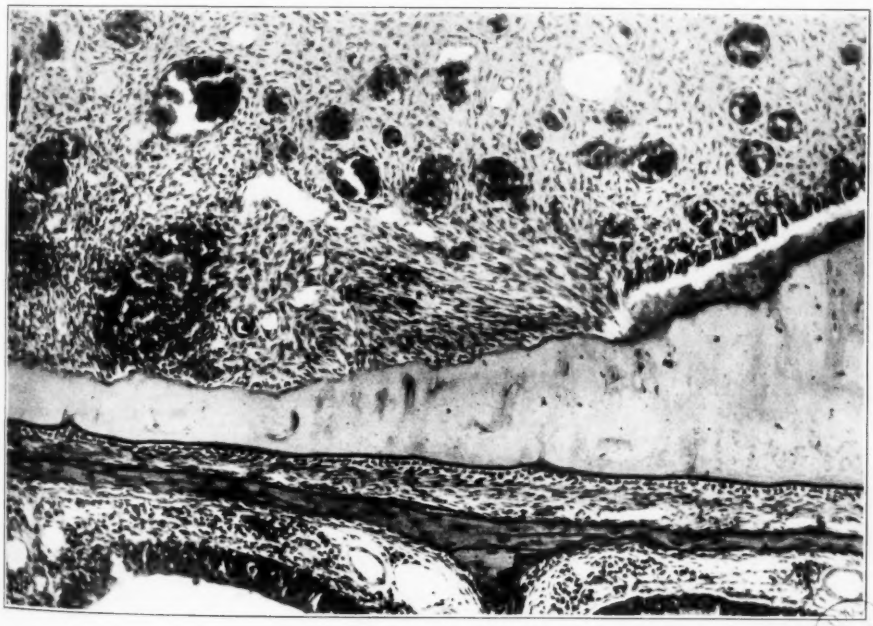

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PLATE 42

FIGS. 9 and 10. Rat 103. Sections of both upper incisors through formative ends. 180 days on vitamin A deficient diet. Fig. 9 shows particularly well the folding of the dentine which makes possible the inclusion of ameloblasts. Both photographs show clusters of proliferated odontoblasts and in Fig. 10 the gland-like formations due to ameloblasts free in the pulp at A. For high power see Figs. 11 and 12. × 46.2.

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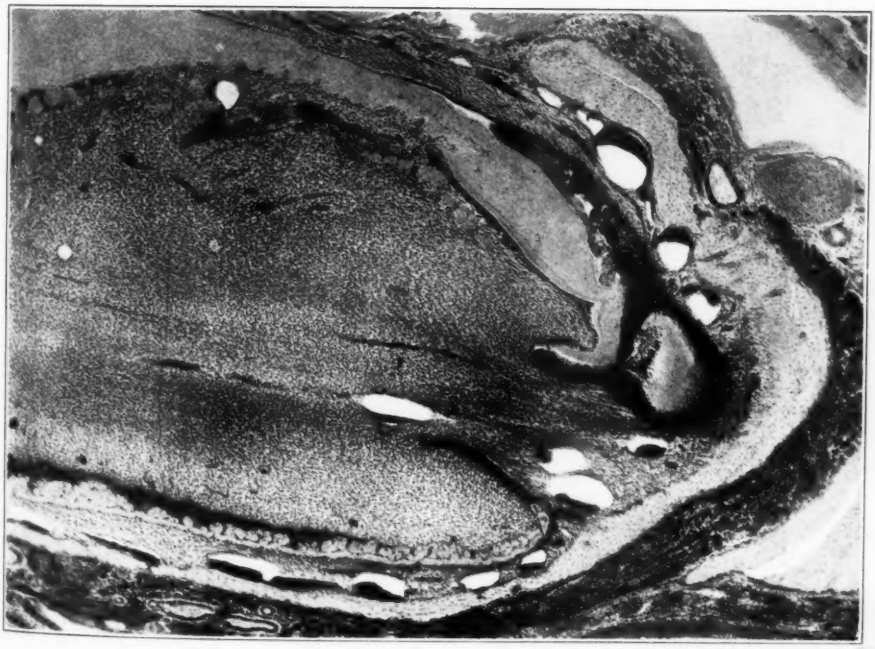

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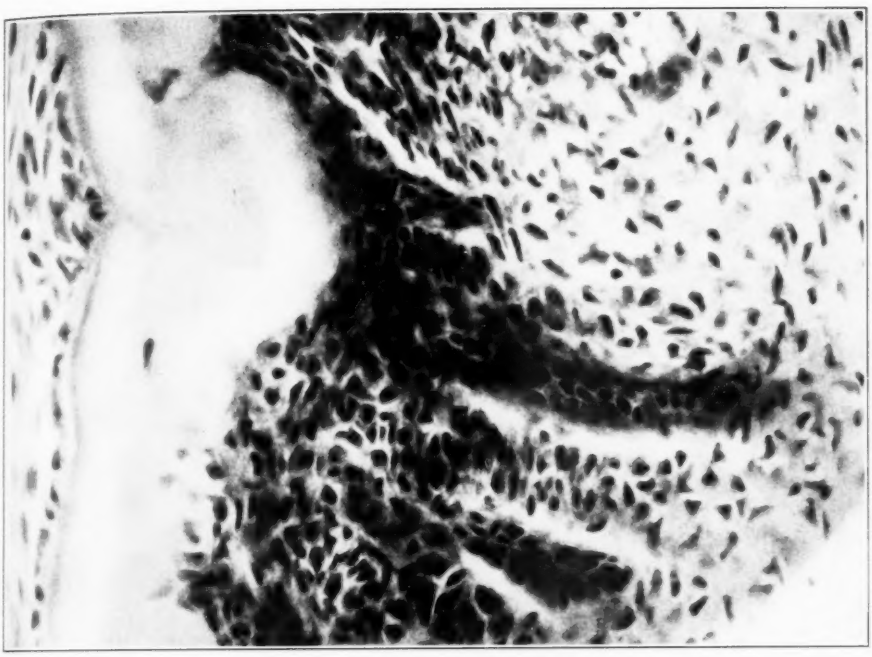
Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

PLATE 43

FIGS. 11 and 12. High power details of Fig. 10 showing inclusions of atrophied ameloblasts in the pulp. In Fig. 11 osteodentine is being formed by pulp cells external to the inclusions of ameloblasts. × 588.



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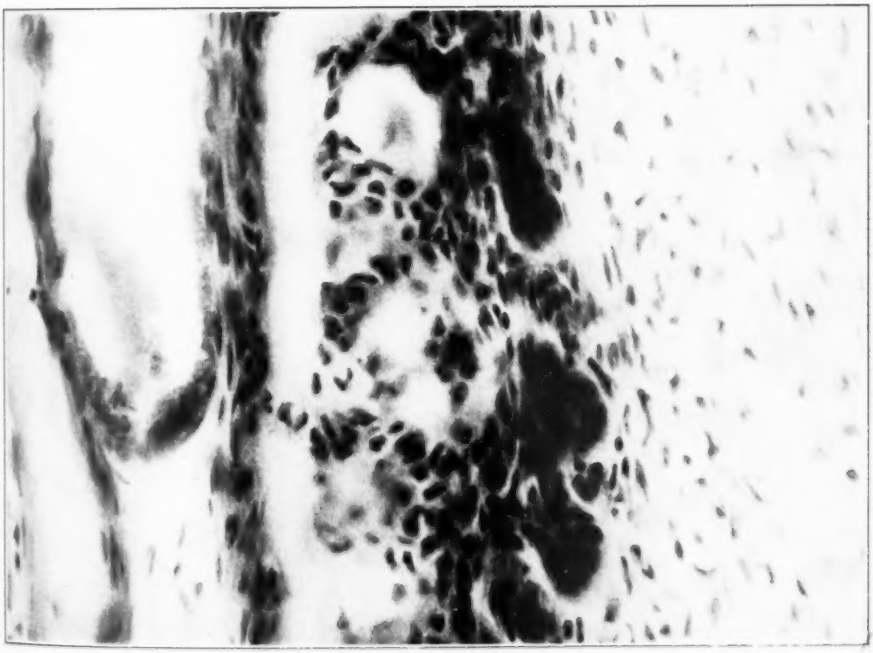

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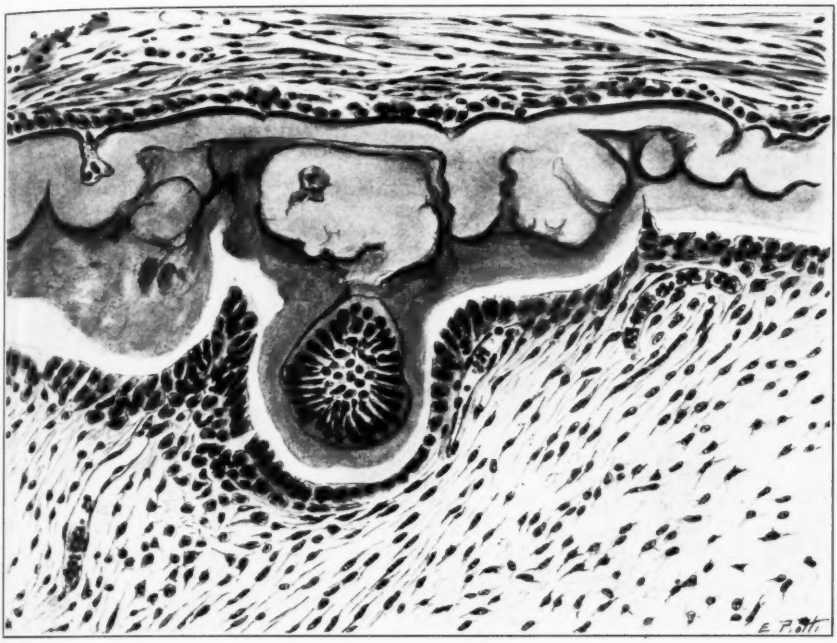
PLATE 44

FIG. 13. Rat 412. Upper incisor. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. At A and B are ameloblast inclusions in the dentine. Newly formed dentine has filled spaces between the folds of dentine and has surrounded the inclusions of ameloblasts. For high power detail see Fig. 14. The restoration of odontoblasts is shown in both Figs. 13 and 14. Heavy calcification of the dentine on the labial side of the tooth is shown, a usual response to restoration of vitamin A. × 46.2.

FIG. 14. Drawing. A detail from Fig. 13 at B. Shows dentine formation about ameloblast inclusion as a result of restoration of vitamin A to diet. The odontoblasts have not yet recovered normal size and form, yet much dentine has been deposited and calcified. The irregular outline of the folded dentine of the deficiency period is less deeply calcified than the dentine deposited during the recovery period. The ameloblast inclusion was probably not surrounded by dentine during the deficiency period. × 300.



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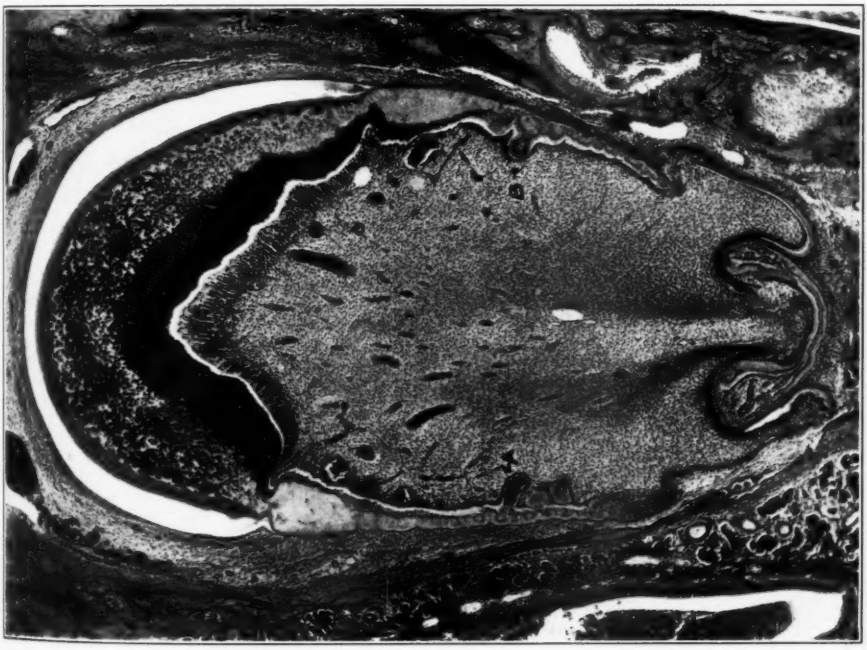

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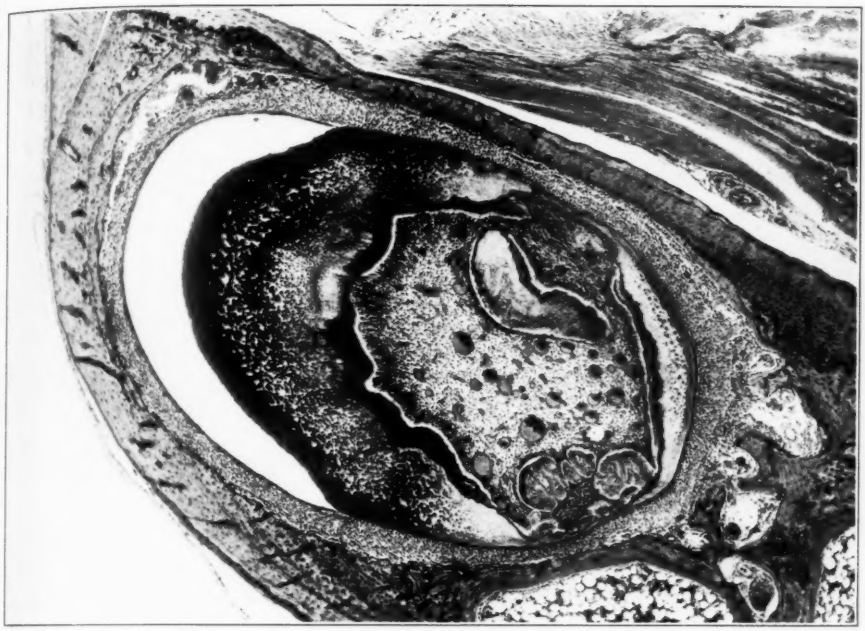
Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

PLATE 45

FIG. 15. Rat 417. Upper incisor. 187 days on vitamin A deficient diet followed by 7 days with addition of butter fat. To show gland-like formation of ameloblast inclusions in pulp in recovery from atrophy. Note mitotic figure. × 588.

FIG. 16. Rat 412. Lower incisor. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. To show growth of denticles in consequence of restored diet. Heavy calcium deposits in old dentine. × 46.2.



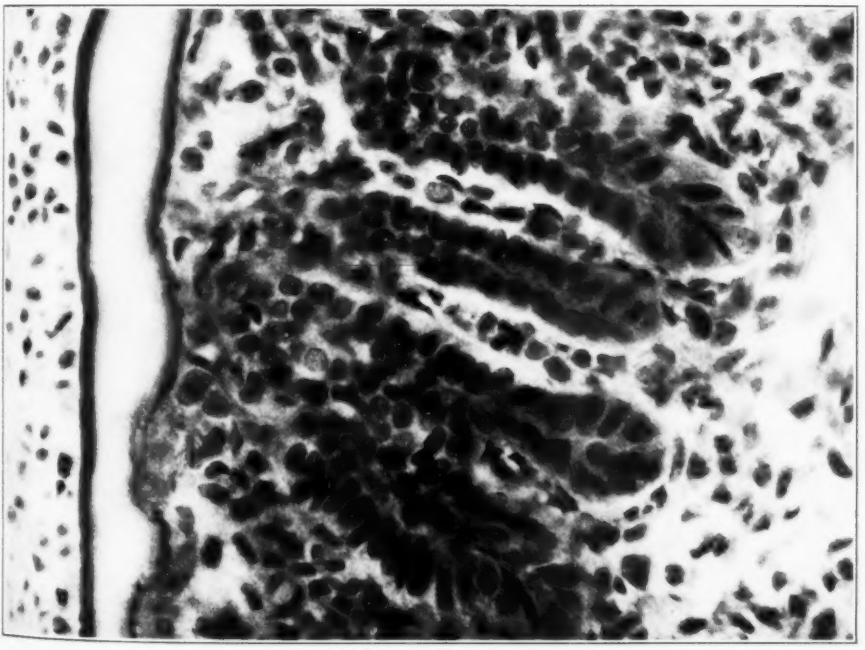
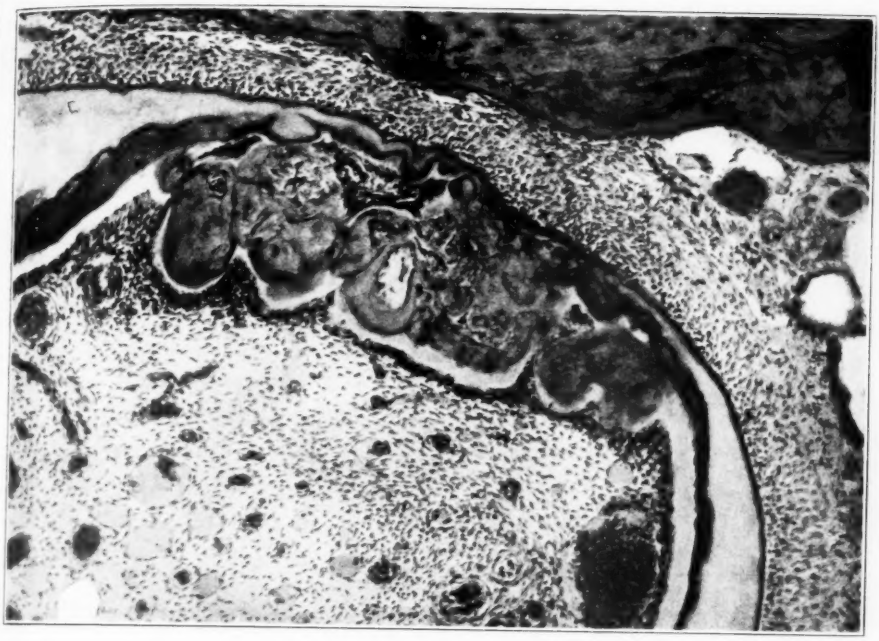


PLATE 46

FIG. 17. Rat 401. Upper incisor. 161 days on vitamin A deficient diet followed by 22 days with addition of butter fat. Denticles of both types showing large deposits of dentine formed since restoration of the diet. × 46.2.

FIG. 18. High power detail of Fig. 17 showing that the odontoblasts, though functionally active, are not yet completely restored in morphology. Inclusions of ameloblasts have disappeared. × 147.



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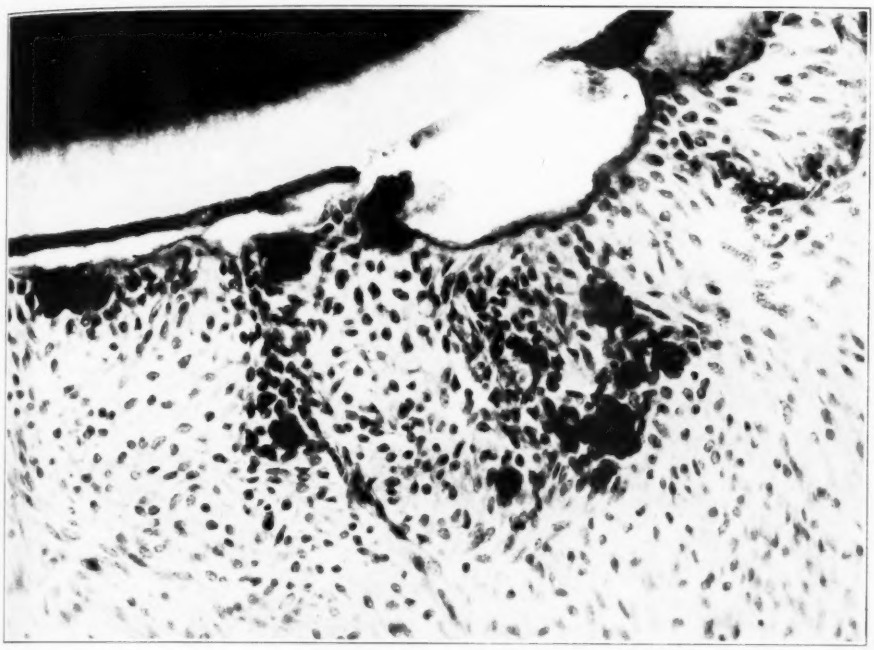
Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

PLATE 47

FIG. 19. Rat 412. Upper incisor. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. To show odontoblasts formed about ameloblast inclusions in the pulp. The former have deposited a considerable amount of dentine. The latter are atrophic. × 588.

FIG. 20. Guinea pig 142. 128 days on experimental diet without source of vitamin A other than 4 cc. of orange juice daily. Shows nearly complete atrophy and disappearance of enamel organ with calcified deposits in the atrophic remains of the enamel epithelium and external to it. × 294.



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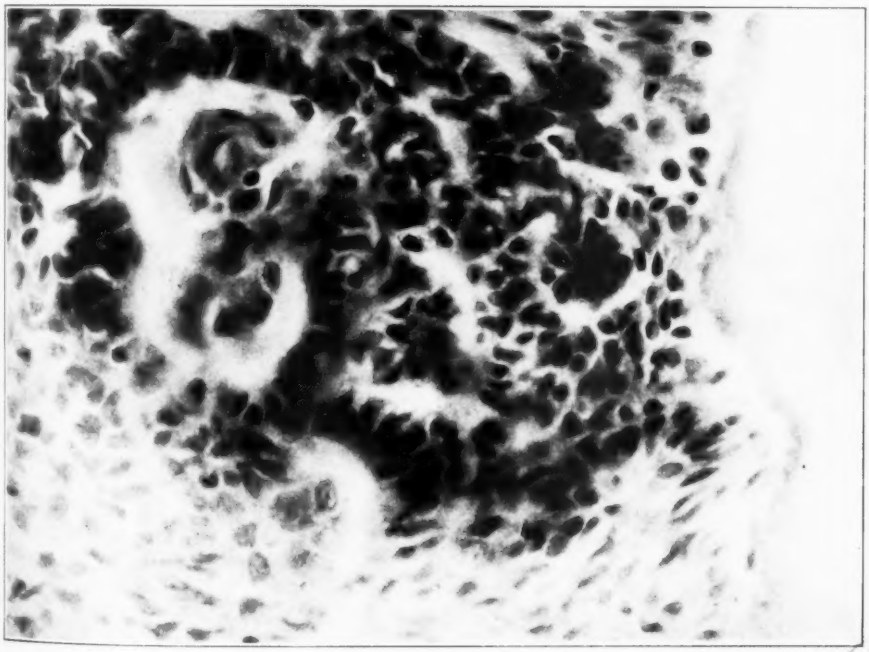

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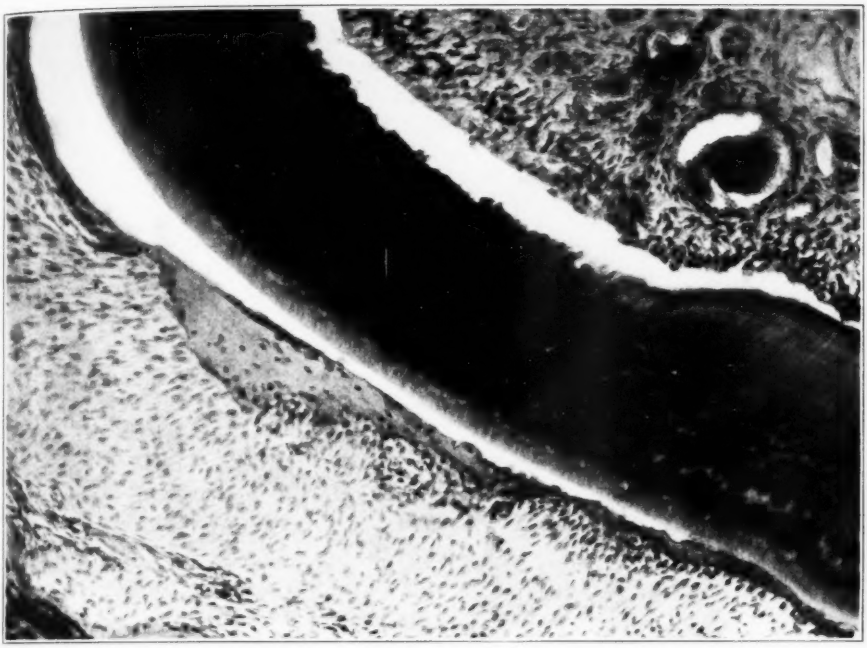
PLATE 48

FIG. 21. Guinea pig 142. Lower incisor. Extreme atrophy and calcification of enamel organ. × 294.

FIG. 22. Guinea pig 38. 91 days on experimental diet without source of vitamin A other than orange juice, of which it received 20 cc. daily for 56 days, 8 cc. daily for 21 days and 4 cc. daily for 24 days. Ossification of enamel organ. × 147.



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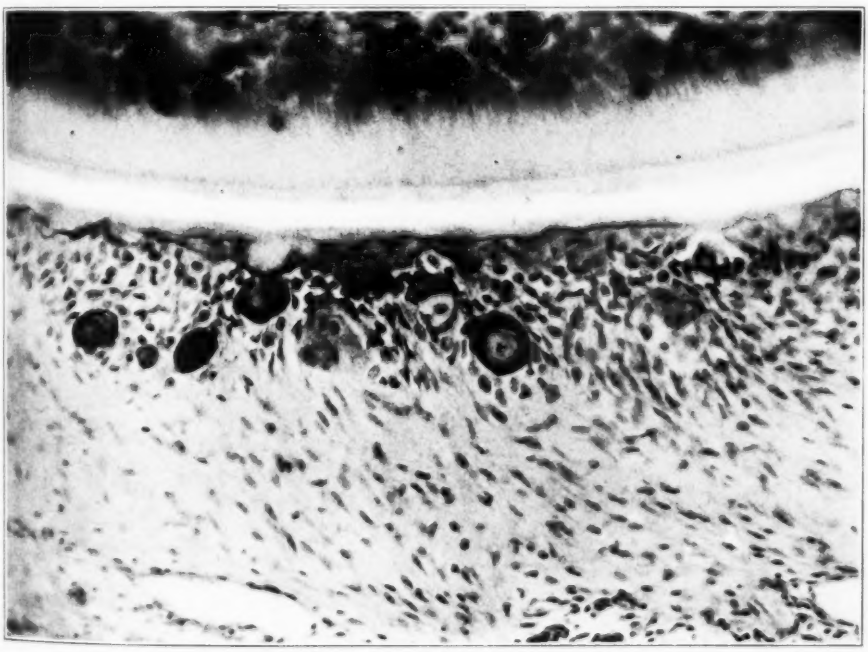


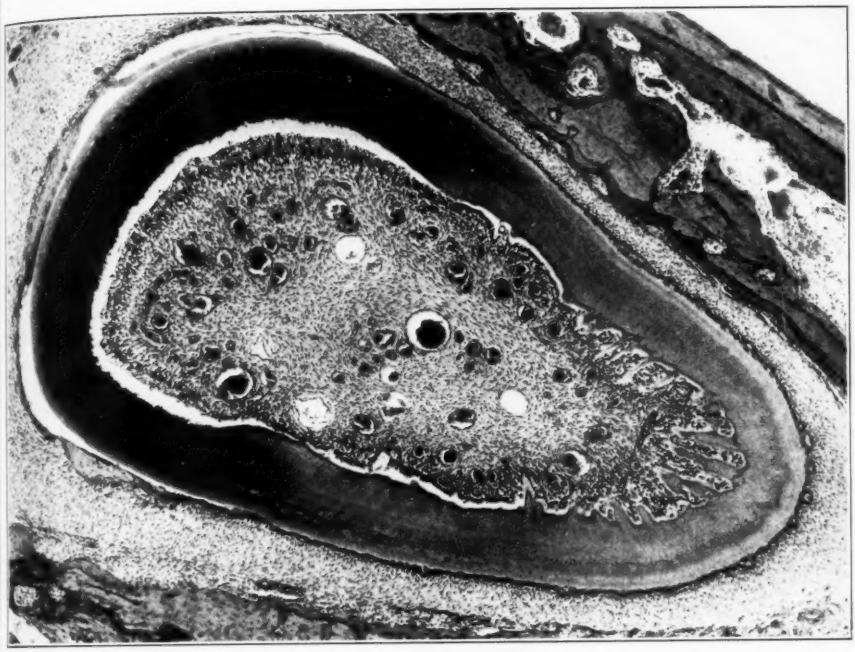
21

Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

PLATE 49

FIG. 23. Guinea pig 38. Same preparation as Fig. 22. Another region. At each end of the bony plaque and external to it, remains of the enamel epithelium can be seen. × 147.

FIG. 24. Guinea pig 38. Lower power. Same preparation used for Figs. 22 and 23. Shows ossification of the enamel organ, cementicles, and on the lingual side, numerous ingrowths of osteodentine. × 46.2.



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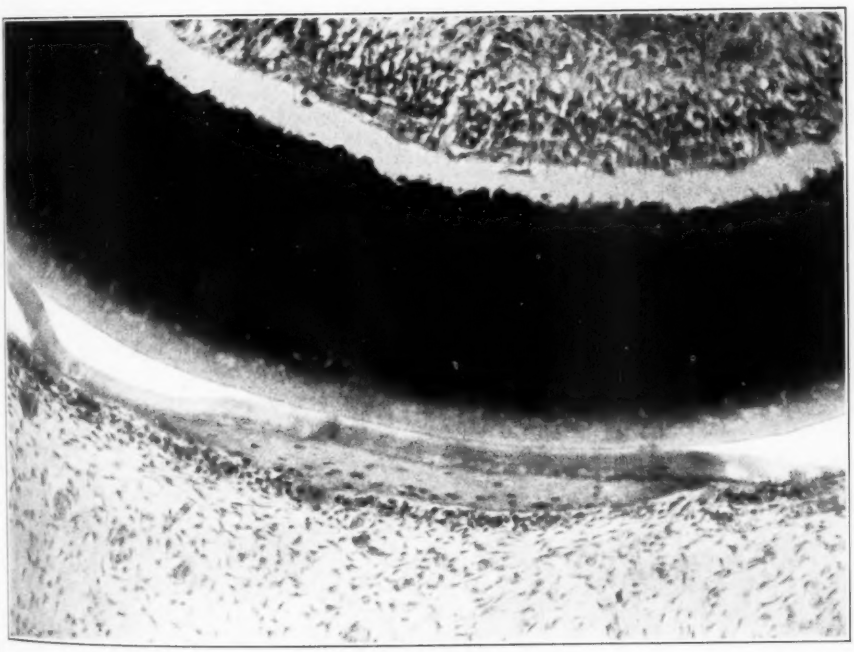

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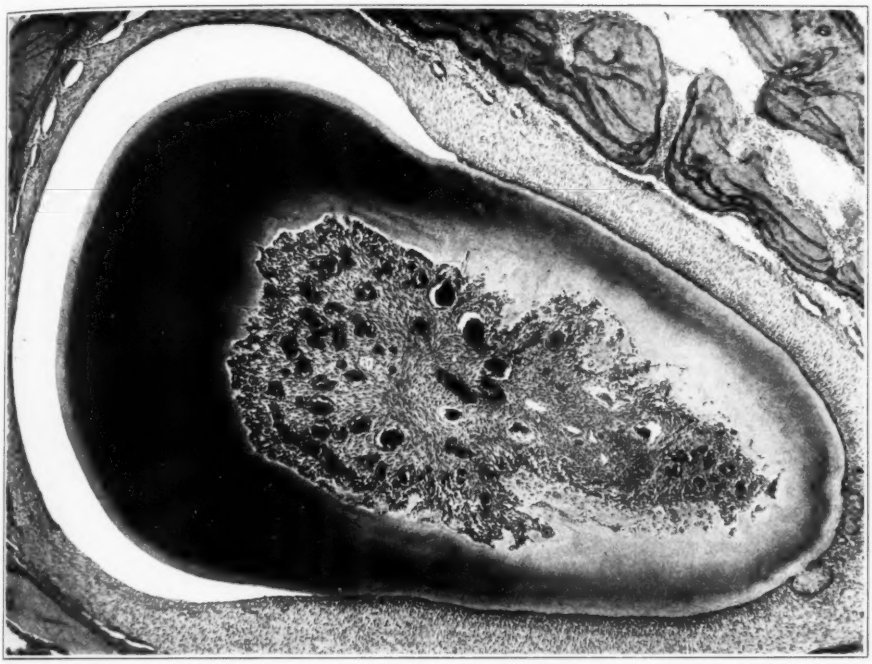
PLATE 50

FIG. 25. Guinea pig 41. Upper incisor. 209 days on experimental diet without source of vitamin A other than orange juice. For 207 days it received 20 cc. of orange juice daily, 10 cc. daily for 15 days and 5 cc. for the last 75 days. Shows nearly complete atrophy of enamel organ and of odontoblasts, except on labial side of tooth. In the guinea pig, in contrast to the rat, atrophying odontoblasts because of depolarization act like osteoblasts and deposit matrix centrifugally. The excrescences of osteodentine, or denticles, are of considerable size. × 46.2.

FIG. 26. Guinea pig 46. 158 days on experimental diet without source of vitamin A other than orange juice, of which it received 20 cc. daily for 28 days and 8 cc. daily for 130 days. Note cementum outgrowths, cementicles and atrophy of odontoblasts with osteodentine formations. × 46.2.



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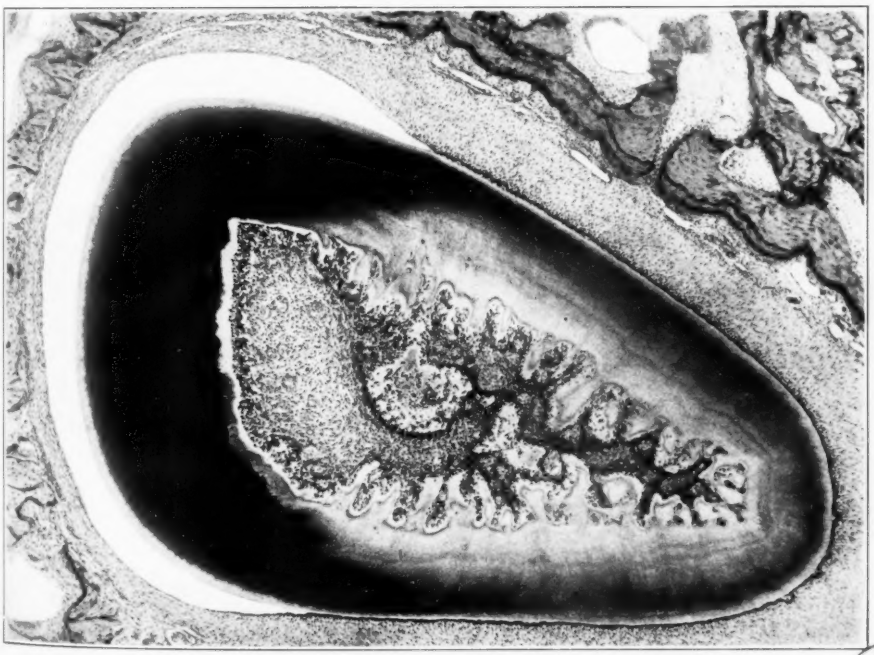

25

Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

SPONTANEOUS RUPTURE OF THE HEART*

REPORT OF 40 CASES IN PORTLAND, OREGON

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REVIEW OF LITERATURE

The great William Harvey [1] in 1647, besides describing the circulation of the blood, recorded the earliest observation of cardiac rupture. His patient was a nobleman who often suffered with paroxysms and oppressive pains in the chest. Finally, "the disease growing more violent, he became cachectic and dropsical, and at length, being violently oppressed in one of the paroxysms, he died. In this patient, by reason of the circulation of the blood being obstructed from the left ventricle into the artery, the very substance of the left ventricle itself, which seemed to be pretty thick and strong, had been ruptured and perforated with a large opening, where it had discharged its blood; for the foramen was sufficiently large to admit a man's finger."

In 1765 Morgagni,[2] the eminent morbid anatomist and president of the University of Padua, collected 10 cases and added an observation of his own, "made upon a most powerful monarch." It is of a peculiar grim interest that Morgagni himself eventually suffered the same type of death.

Morand [3] in 1732 who, Morgagni says, had "written and collected more than any other person ever did yet on the subject of rupture of the heart," was probably the first to associate this condition with myocardial softening. Then came the observations of Verbrugge, Portal and Laennec.[4]

In 1757 Galeati [5] published the first account of an aneurysm of the left ventricle and described rupture of this into the pericardial sac.

Blaud [6] in 1820, referred to by Cruveilhier as "that estimable observer, who, in a small country town has so often paid his tribute to science" collected 4 cases of rupture, and attributed them to a soft

* Received for publication December 27, 1932.

gelatinous degeneration of the myocardium. Likewise Fleury [7] in 1812, Rostan [7] in 1820, Dézeimeris [8] in 1839, and Cruveilhier [9] in 1849 made observations that at least contributed to the knowledge of this cardiac accident.

In Dublin arose a school of writers including such noted names as Cheyne [10] in 1818, Robert Adams [11] in 1827, Townsend [12] in 1832, Smith [13] in 1836, Stokes [14] in 1846, Bellingham [15] and Kennedy [16] in 1849, who designated the senile or gelatinous degeneration described by the French writers by a different name — fatty degeneration — and attributed cardiac rupture to this cause. Adams described a rupture of the left ventricle in a gentlewoman over 60 years old, of corpulent habit and "confined bowels" who was attacked with pain in the epigastrium. Fomentations and liniments on the abdomen, pills containing blue pill and calomel, then a further purgative draught of infusion and tincture of senna and Rochelle salts, then a terebinthinate enema followed by "blooding," all failed to relieve her and she died suddenly in a fit of vomiting. Autopsy showed rupture of the left ventricle, whose inside was so soft "as to admit of being broken down between the finger and thumb." In England Williams [17] in 1840, and Quain [18] in 1850 followed the same lead but at least evinced the suspicion that coronary narrowing might be the underlying cause.

As early as 1815 one English author, Joseph Hodgson,[19] expressed an opinion that comes close to the modern conception of coronary disease. He reported rupture of the left ventricle where "the degeneration of the structure of the ventricle had in one part proceeded to such an extent that the remaining fibers were unable to resist the force of the circulation." He found the left coronary artery completely occluded by calcareous deposit, but like all others of that period overlooked thrombosis of the coronary arteries.

This early phase of coronary study ended with several noteworthy reviews. In 1857 Elléaume's important Paris thesis [20] collected about 50 spontaneous ruptures up to that time, and in 1871 Barth [21] added 24 more. Many causes had been assigned for heart rupture — most of them erroneously. To be sure several authors had described senile softening of the myocardium and a few, including Barth, had connected this with atheromata of the coronaries. Cruveilhier [22] had in 1850, according to Huchard,[23] even reported coronary thrombosis, without appreciating its full significance. But none had even hinted

at the now recognized sequence of coronary sclerosis — thrombosis, infarction and rupture.

The second period in the study of cardiac infarction and rupture starts probably in 1861 with Malmsten.[24] This Swedish observer was the first definitely to affirm a relationship between coronary thrombosis, myocardial softening and rupture. In describing a rupture of the left ventricle he says: "Microscopically the muscle fibers were here destroyed and replaced by a highly granular detritus, mingled here and there with fatty granules. In places there were small areas, visible even to the naked eye, where only connective tissue remained, buried in the degenerated mass." The anterior branch of the left coronary artery contained an old white thrombus situated just before the entrance of the artery into the degenerated area. "The clot in the coronary artery was undoubtedly old. The arterial obstruction had resulted in a slow softening of the muscular tissue. Finally rupture appears to have occurred slowly." It seems only fair to regard the foregoing as a valid presentation of the concept of infarction.

In 1878 Ménard,[25] a Frenchman, definitely associated coronary thrombosis and rupture of the heart. In 1880 a physician in Boston named Winsor [26] made an epochal contribution. In describing a case of angina pectoris with rupture he reports: "In the vicinity of the rent — the characteristic appearance of the muscle was lost, the muscular fibres being here filled with a granular material, and in many places with minute fat drops." The walls of the left coronary artery were thickened and sclerotic and their lining covered with yellowish white patches. "At one point, about 3 cm. from the origin of the artery, one of these patches had so far protruded into the lumen of the vessel as to cause a coagulation of blood at that point, which coagulation had become adherent to the walls, thereby preventing the flow of blood through the vessel. The portion of the heart in which the rupture had occurred and in which the fibres were found degenerated corresponded to the territory supplied by the branches of this artery." This constitutes probably the first entirely valid presentation of the etiology of heart rupture.

Other corroborative reports appeared about this time. In Germany Weigert [27] in 1880 enunciated the principle of cardiac infarction and was followed by Ziegler,[28] Cohnheim and von Schulthess-Rechburg,[29] and Huber.[30] The interrelation of coronary thrombosis, myocardial ischionecrosis and rupture is further supported by the

writings of Samuelson [31] in 1881, Birch-Hirschfeld [32] in 1883, Leyden [33] in 1884, and Georg Meyer [34] in 1888 in Germany; Poppoff [35] in 1882 in Russia; Mollière and Duplaix [35] in 1883 in France; and in England of Steven [36] in 1887, who mentions other lesser contributions of that time.

René Marie [37] in his noted Paris thesis of 1896 sums up the results of these various writers and concludes that the lesion that determines rupture is not, as Odriozola [38] in 1888 had maintained, a progressive arteriocapillary ischemia, but infarction from sudden thrombosis of an artery.

Since René Marie's time little further progress has been made in the development of the morbid anatomy and pathogenesis of heart rupture. Thrombosis and infarction are accepted as the inciting cause. At present the question arises if we may not now be embarking on a third phase of the problem. Experimentation is being directed toward a solution of the underlying causes of arteriosclerosis, thrombosis and infarction. Also, since there are other sources of heart rupture besides infarction, it is essential to differentiate these in each case.

Incidence of Cardiac Rupture

Cardiac rupture appears never to have been reported up to the time of Harvey's publication of his case in 1647.[1] Probably only 10 instances appeared during the following century, according to Morgagni.[2] In the course of still another century, Elléaume [20] was able to add 47 more. After this the number increased rapidly. Barth [21] in 1871 collected 24 from recent reports in the *Bulletin de la Société d'Anatomie* from 1826 to 1865 and Odriozola [38] in 1888 added 110 more.

The latest tabulations indicating a greatly increased incidence are those of Krumbhaar and Crowell [39] in 1925, who collected from the literature (excluding Elléaume and Barth) 632 ruptures, exclusive of 22 unpublished cases from Philadelphia hospitals, and Davenport [40] in 1928, whose supplementary list of 92 further instances raised the total to 734. This number, while high as compared with previous figures, does not represent an incidence nearly as great as is found in some autopsy services and probably includes but a small fraction of the real total.

The percentage of cardiac ruptures in hospital morgues is always

low, for the obvious reason that such patients are usually not spared long enough to reach a hospital. It will suffice if we cite Krumbhaar and Crowell's [39] figure of 7 ruptures in 16,000 autopsies at the Philadelphia General Hospital; the Munich report of 7 in 13,000, as cited by Georg Meyer [34]; and the Leipzig series of 9 in 8000 (Krumbhaar). De la Chapelle [41] has more recently recorded 20 cases of rupture among 15,000 autopsies in the medical examiner's service in New York City. A notable exception among hospital statistics is the incidence of 0.86 per cent of cardiac ruptures among 2374 autopsies, reported by Beresford and Earl [42] in 1930 from the Tooting Bec Hospital in England, an institution whose patients are largely senile persons suffering from mental disorders. To Beresford and Earl also belongs the credit for reporting the largest number of cardiac ruptures thus far studied by a single group.

Our figure of 40 ruptures of the heart among nearly 7000 autopsies (0.57 per cent) is in sharp contrast to all figures heretofore obtained, with the exception of Beresford and Earl's.[42] Its explanation admits no doubt. Most of these patients died without medical attention, 27 reaching the coroner's morgue. Of the remaining 13 examples 3 were from private practice and 10 died in the hospital. Werley,[43] in a smaller series, reports an incidence of cardiac rupture equal to that of our coroner's group. He encountered 3 ruptures among 200 autopsies in El Paso during two years (1.5 per cent).

Etiology and Classification

There was a period when it was considered ridiculous to hold that cardiac rupture could be caused by any other agent than fatty degeneration of the heart. Thus Quain [18] deplored the fact that Corvisart and Cruveilhier doubted the rôle of fatty change in producing myocardial softening.

The research of a century has now demonstrated the importance of coronary arterial disease as the source of heart rupture. Most authors, perhaps too freely, admit that the only factor worthy of consideration is coronary occlusion. They assume, often without ample histological verification, that the sequence of events is invariably as René Marie [37] maintained in 1896, — coronary sclerosis, thrombosis, infarction { rupture, aneurysm, myofibrosis.

They are probably quite correct in maintaining that this order usually holds, but a suspicion arises that they are on occasion, by blindly following the accepted doctrine of the preceding age, missing an opportunity to demonstrate other causes of rupture.

It is reasonable to suppose that rupture may be induced by any occlusion of coronary arteries, especially recent occlusions, with or without anatomical evidence of infarction, but it is equally plausible that the heart wall may break if its resistance is lowered by disease of the myocardium independently of disease of the nutrient arteries. It is also possible to find myocardial changes, such as fatty infiltration or fatty degeneration, coincident with arterial obstruction.

We shall therefore attempt to classify cardiac rupture, first as to site and then according to the various causes found in the more authentic reports.

Rupture may occur:

(*a*) Through the ventricular wall externally.
(*b*) Through the auricular wall externally.
(*c*) Through a portion of the thickness of the external wall, by coronary rupture and dissecting hemorrhage.
(*d*) Interventricularly or interatrially through the septum.
(*e*) Through the heart wall internally or externally by a dissecting aneurysm of the aortic base.
(*f*) In papillary muscles.
(*g*) In valve cusps.

Cardiac ruptures usually occur in the left ventricle, less often the other chambers, at the site of recent infarction or occasionally in old cicatrized areas with fresh necrosis. In Davenport's [40] tabulation the left ventricle was the seat of rhexis in 566 (79.8 per cent); the right ventricle in 76 (10.7 per cent); the right auricle in 38 (5.3 per cent); and the left auricle in 13 (1.8 per cent). Ruptures of the anterior left ventricular wall predominated with a ratio of 3 to 1 over the posterior surface.

Other seats of rupture are: The papillary muscles, 9 instances in all — Corvisart [44] 1812; Bertin [45] 1824; Dennig [46] 1909; Wankel [47] and Teacher [48] 1911; Fischer [49] 1922; VonGlahn and Horowitz [50] 1923 (coronary thrombosis case); Eichhorst [51] 1921, the last being reported as due to syphilis (as was also the case reported by Spalding and VonGlahn [52] the same year, with demonstration of spirochetes); the interventricular septum, usually, though not always, in the pars

membranacea — see Markham [53] 1859, Pergami [54] 1876, Crowther [55] 1880, Meyer [34] 1888, Youmans [56] 1921, Faulkner, Marble and White [57] 1924, Martin and Waugh [58] 1925, Werley [43] 1925, Hyman [59] 1930, Nickson and Benson * 1931 and our 2 cases reported here, about 26 in the literature; and the valves, represented by Howard [60] and Thayer [61] 1925. Many more might also be cited.

Dissecting aneurysms are made the subject of a detailed thesis with long bibliography by Vestberg [62] in 1897, who collected 59 cases besides his own single one. He classifies them as parietal, septal, valvular, and interparietal, the point of predilection being, however, at the junction of heart and aorta. From this point of origin the hemorrhage may dissect through the heart wall and rupture into the pericardial sac or into an auricle. We have had one which, starting in a sinus of Valsalva, dissected through the interauricular septum and bulged into the left auricle without rupturing. Our other aneurysm started similarly, bulged into the right auricle, but finally ruptured anteriorly into the pericardial sac. Claytor [63] had a similar case.

The coronary arteries themselves may rupture into the myocardium, and thence usually into the pericardial sac, as in the reports of Galliardi [64] 1840, Odriozola's [38] 5 examples in 1888, Dock,[65] and Rochester [66] 1896, LeCount [67] 1918, Kalyanvala [68] 1919, Kesteven and Verco [69] 1920, Jones-Evans [70] 1922, Benson and Hunter [71] 1925, and Lewis [72] 1925. Coronary aneurysm is reported by Odriozola,[38] LeCount [67] and others.

The pathogenesis of rupture, according to the best reports available, may be classified as follows:

1. Cardiac infarction due to thrombosis or embolism is admittedly the commonest cause and appears throughout the modern writings. Arteriosclerotic disease of the coronary arteries is the basis of most thromboses, while embolism may arise from distant pyemic abscesses, from endocarditic vegetations (Huber [30] 1882, LeCount [67] 1918); from floating thrombi, as reported by numerous authors, or from atheromatous plaques loosened off from the lining of an artery and carried along in the circulation. Of this latter type Panum,[73] in 1862, reported the well known case of Thorwaldsen, who died suddenly in a Copenhagen theater. Dock [65] 1896, and LeCount [67] 1918, reported similar cases and we are reporting another. Allbutt [74] 1921, also discusses accidents of this kind in syphilitic arteries.

* Unpublished case.

2. Cardiac aneurysm is occasionally the seat of rupture, but usually only in instances in which a fresh coronary occlusion supervenes. We are reporting some cases of this type.

3. Gummas with rupture were, according to Georg Meyer [34] first described by Oppolzer, but they also have nearly disappeared from the literature as a cause. Diffuse syphilitic myocarditis, on the other hand, is occasionally blamed, probably in certain instances with truth, as in the three examples of Harlow Brooks [75] in 1921. Aubertin [76] in 1924, who gives a good summary of the pathology of rupture, cites exceptional instances of rupture without localizing lesions, but with chronic myocarditis or fatty degeneration of the myocardium. It would seem that such cases might well be stained for spirochetes. In this connection the reader is referred to Warthin [77] 1918, 1925.

4. Authentic reports of rupture of tuberculous lesions are represented by Korybut-Daszkiewicz [78] 1922, Oudendal [79] 1923 and Brandi [80] 1931. Tuberculosis of the myocardium is occasionally reported in cases without rupture. Of special interest are two reports by Swiss investigators, Massini [81] and Lüscher [82] in 1921, of masked tuberculous myocarditis with animal verification.

5. Endocarditis appears frequently in the older writings as a cause of rupture, as in Böttger's 3 cases, cited by Meyer,[34] but is usually to be discounted. The more reliable recent reports include those of Claytor [63] in 1923, Locke [83] in 1925, and a few others. Claytor's appears to be in reality a dissecting aneurysm with rupture and we are including a similar case in our series due to *Streptococcus viridans*. Nickson and Benson * have also observed an instance of perforation of the membranous interventricular septum due to erosion by subacute *Streptococcus viridans* endocarditis of the tricuspid valve. Endocarditis as a direct cause of rupture is often open to doubt unless minute examination of the coronary arteries for embolism has been made.

6. Meyer [34] in 1888 cited what he thought was the only then known case of rupture of the heart due to echinococcus cysts, that of Dézeimeris [8] in 1834, but he evidently overlooked the report of Crowther,[55] who in 1880 described a perforation of the interventricular septum by rupture of a hydatid cyst. Echinococcus of the heart without rupture has also been reported; thus Hynd [84] in 1924, in a case of sudden collapse and death with respiratory failure, found that

* Unpublished case.

in the right ventricle a subendocardial cyst the size of a hen's egg had emptied its contents, consisting of smaller cysts, which proved microscopically to be echinococcal. Several cysts were found in the branches of the pulmonary arteries.

7. Malignancy is sometimes mentioned as a cause of rupture, but Meyer [34] failed to find any ruptured neoplasms in the reports up to 1888, and Jores [85] in 1924 discovered none in the recent literature. Krumbhaar and Crowell,[39] however, include a melanotic sarcoma in their tabulation, although they do not print the reference. Weiss [86] in 1880 also reported a large round cell sarcoma of the right auricle which had broken through the interauricular septum and produced metastases in the lung and other foci.

8. In a general treatise such as this, attention should be paid to the fact that cardiac rupture is not confined to the human species. Thus Meyer [34] says that it occurs in horses and cattle, and that Virchow and Hirsch's Jahresberichten reports 6 cases in animals in 10 years. Lewis [72] also writes on rupture in animals.

Besides the various inciting causes listed above we should mention certain predisposing factors. Hippocrates, Celsus and Harvey referred to the frequency of sudden death in obese individuals, and many writers, beginning with Morgagni, have observed that rupture also occurred with high incidence in the obese.

Likewise adipose deposits in the epicardium have been noted by many as a predisposing cause. Fatty degeneration is, on the other hand, open to doubt as a causative factor, being probably rather an incidental by-product of infarction or, as Warthin [77] has pointed out, of syphilitic myocarditis. In fact Master [87] has shown the presence of fatty granules in normal heart muscle cells. The full stomach is claimed by various observers as a common predisposing factor in cardiorrhexis.

Silverthorn [88] mentions that rupture may occur in the absence of exercise, even during sleep, but Krumbhaar and Crowell [39] cite MacWilliams as authority for the statement that sleep is not always free from heart strain, and that during dreams or restless sleep the blood pressure may be increased and changes of rhythm such as heart block may appear. In most cases rupture follows some form of physical exertion.

When all these factors are properly evaluated there is one which stands out above all others, namely, coronary occlusion, with or

without anatomical evidence of infarction. The obstruction of arteries may be a slow one from sclerotic closure of coronary orifices, atheromata, arteriosclerosis, syphilitic arteritis, various chronic endarteritides; from pressure from without, notably in encasement by myocardial or other scars and in encroachment of dissecting aneurysms. Sudden obstruction of arteries, which is more important, is due to thrombosis, less often embolism; rarely dissecting coronary aneurysm; acute arteritis and endarteritis, and, as cited by Herrick [89] in 1912, has even been caused by surgical tying-off of a coronary artery. This last was followed by infarct but not rupture. The commonest of all is a mixed type, the chronic arteriosclerotic narrowing complicated by sudden thrombosis or even embolism.

Case Reports

The present report embraces 40 ruptures of the heart, of which 2 are perforations of the interventricular septum, 3 are ruptures of coronary arteries into the pericardial sac, and another an anterior cardiac rupture of a dissecting aneurysm of a sinus of Valsalva. We include, in distinction to de la Chapelle,[41] all breaks in the continuity of any portion of the heart, the base line of the aortic valve being the upper boundary of what may be termed the heart. We, therefore, include ruptures, such as interventricular, which do not result in extravasation of blood into the pericardial sac. We have not, however, burdened our series with erosions of the chordae tendineae resulting from endocarditis.

Of these 40 ruptures, 35 have been encountered among a total of 5896 autopsies performed by the pathology staff of the University of Oregon Medical School, 2112 of which were done for the coroner of Multnomah County, the remaining 3784 private and hospital cases. Among the 35 ruptures in the Medical School group 27 were on the coroner's service. Of the remaining 13 private cases 10 died in hospitals, 5 of these being encountered among 873 autopsies performed by one of us (C. H. M.) at the Good Samaritan Hospital. This gives an incidence of 1.27 per cent cardiac ruptures in our coroner's series and 0.28 per cent in our private cases. For permission to publish Case 37 we are indebted to the U. S. Veterans' Bureau, a more extensive report of which has already appeared.[90] In a previous preliminary tabulation of coronary disease,[71] we have already briefly mentioned

Cases 1 to 7, also 6, 7, 8, 30, 32, 33, 35 (Table I). In classifying our 40 cardiac ruptures as to the underlying morbid anatomy we find that they fall into several groups.

Group A. Rupture of Heart Due to Coronary Thrombosis (25 Examples)

Group A includes 25 instances of rupture of the heart through recent infarcts due to coronary thrombosis. Nearly two-thirds of all our cases fall in this category, a not surprising finding since the literature on cardiorrhexis is replete with examples of the type included in this class.

We shall therefore make only brief mention of our cases of rupture through acute infarcts and give more attention to the less common forms. The essential pathological data of Group A are listed in Table I. Seventeen of the 25 cases came to coroner's autopsy, 3 were private cases and 5 died in the hospital. In 16 instances death occurred suddenly and without forewarning, 9 others had a history of pain or other symptoms referable to a previous coronary attack. In 5 cases death came during or shortly following physical exertion: Case 2 after playing golf, Case 4 at the completion of his train run, Case 8 while working in the kitchen, Case 11 while shovelling snow, and Case 14 while painting a sign.

As shown in Table 1 there was in each of the 25 instances of rupture of recent infarct, except in Case 11 where the record was lost, a thrombus in the coronary artery supplying the area of rupture. Even in Cases 5, 10, 12 and 17, all of which appear to be syphilitic, coronary thrombosis and infarction were found. There seems to be no question then that these 25 examples constitute classical coronary thrombosis resulting in cardiac infarction and rupture. In this group approximately two-thirds were sudden deaths in the first coronary accident without previous symptoms referable to coronary occlusion.

Group B. Recent Infarct Superimposed on Old Cardiac Aneurysm (4 Examples)

CASE 26: (Path. No. 2225). Mrs. C. B., aged 65, a housewife, called her physician 17 days preceding her death, and complained of what she thought was la grippe, with aching, general malaise, and slight dyspnea and cough. The temperature at onset was 100° F, rising a few days later to 101°. In the course of her fatal illness precordial pain developed, with smothery feeling. Death came suddenly and unexpectedly, the only noteworthy sign being the terminal gasp.

Autopsy: This record was lost, but the heart was preserved. The apical portion of the left ventricle is thinned out to form an aneurysm, and gross evidence of fresh infarction is present in this, with dissecting hemorrhage and resulting rupture at the apex.

Histology: The microscopic picture is that of healed aneurysm with evidence of fresh infarction, especially in the margin of the aneurysm. Microscopic examination of sections of the coronary arteries discloses a thrombus with early organization in a branch of the left marginal artery.

Pathological Diagnoses: Rupture of recent infarct of left ventricle at site of old cardiac aneurysm; fresh thrombosis of branch of left marginal artery.

CASE 27: (Path. No. 9042). O. S., aged 71, housewife, died suddenly without medical attention and was subjected to a coroner's autopsy. No history was obtained.

TABLE I

Ruptures of Heart

Group	Case No.	Pathology No.	Sex	Age	Occupation	Weight of heart	Rupture	Cardiac lesion	Coronary occlusion			Myocardium				
									Fresh thrombus	Organized thrombus	Coronary syphilis	Fatty infiltration	Fragmentation	Syphilis	Gall-stones	Obesity
A	1	2137	M	*yrs.* ..	....	*gm.* 270	Left ventricle	Recent infarct	A.I.V.							
	2	5747	M	50+	Business man	400	" "	" "	A.I.V.			Both ventricles				+
	3	6961	M	81	Laborer	500	" "	" "	L. circumflex			"	+			±
	4	9126	M	68	Conductor	480	Coronary artery and left ventricle	" "	" "			"				+
	5	9251	M	50	Quarryman	300	Left ventricle	" "	A.I.V.		+			+		+
	6	9879	M	65	Farmer	370	" "	" "	A.I.V.			L. ventricle	+		+	
	7	11209	M	86	Retired	450	" "	" "	L. circumflex							
	8	12981	F	60	Housewife		" "	" "	R. coronary A.			Both ventricles			+	+
	9	15393	M	75	Gardener	350	" "	" "	R. coronary A.	A.I.V. and R. coronary A.	±	"	+			
	10	15636	M	54	Photographer		" "	" "	A.I.V. branch		+			+		
	11	15839	M	68	Candymaker		" "	" "	*	*	*	*	*	*	*	*
	12	16362	M	75?	Canner	580	Coronary artery and left ventricle	" "	A.I.V.**		+			+		

	13	18785	F	73	Housewife	400	Left ventricle	" "	A.I.V.		±				+	+
	14	19013	M	77	Painter	275	" "	" "	A.I.V.	A.I.V.	±					+
	15	20030	F	75	Housewife	400	" "	" "	L. circumflex							
	16	20805	M	70	Laborer	440	" "	" "	A.I.V.							
	17	21004	M	62	Laborer	430	" "	" "	L. circumflex		+***					+
	18	22–1–30	F	79	Housewife	360	" "	" "	A.I.V. branch	A.I.V. branch					+	
	19	132–3–30	M	65	Theater manager		" "	" "	L. circumflex							
	20	178–4–31	M	60	Unknown		" "	" "	" "	L. circumflex						±
	21	428 GSH	M	60	Physician	450	" "	" "	R. coronary A.	A.I.V.						+
	22	706 GSH	M	52	Salesman		" "	" "	L. circumflex							+
	23	846 GSH	F	56	Housewife	300	" "	" "	R. coronary A. L. circumflex							
	24	307–7–32	M	69	Truck driver	470	Left and right ventricles	" "	R. coronary A.	A.I.V.						±
	25	317–7–32	M	78	Retired	550	Left ventricle	" "	A.I.V.							
B	26	2225	F	65	Housewife		" "	Aneurysm and infarct	L. marginal							
	27	9042	F	71	Housewife	380	" "	" " "	" "	A.I.V.				+			+

A.I.V. = Anterior interventricular branch of left coronary artery.
L. coronary A. = Left coronary artery.
L. circumflex = Left circumflex coronary artery.
L. marginal = Left marginal coronary artery.
R. coronary A. = Right coronary artery.

* Heart lost before description completed.

** Thrombosis of A.I.V. and branches with dissecting hemorrhage of these vessels.

*** Syphilis of aorta.

TABLE I (*continued*)

Group	Case No.	Pathology No.	Sex	Age	Occupation	Weight of heart	Rupture	Cardiac lesion	Coronary occlusion			Myocardium			Gall-stones	Obesity
									Fresh thrombus	Organized thrombus	Coronary syphilis	Fatty infiltration	Fragmentation	Syphilis		
						gm.										
B	28	11716	F	65	Housewife		Left ventricle	Aneurysm and infarct	A.I.V.	A.I.V.					+	±
	29	13543	M	56	Physician		" "	" " "	A.I.V.						+	±
C	30	10160	M	76	Watchman	390	" "	Septic infarct	Multiple emboli	L. marginal		L. ventricle				+
	31	13414	M	70	Timberman		Both ventricles	Recent infarct	Embolism, R. coronary A.	R. coronary A.					+	±
D	32	6093	M	87	Sailor	500	Left ventricle	Old infarct		A.I.V.						
E	33	10923	M	60	Shirtmaker	450	Interventricular septum	Septic infarct	R. coronary A.	L. coronary A.						+
	34	12529	M	81	Druggist	490	" "	Old infarct	A.I.V.		+			+		
F	35	10725	M	45	Fireman	500	Saccular aneurysm of left ventricle	Myofibrosis								+
G	36	112 GSH	F	50	Teacher		Dissecting aneurysm	Mycotic aneurysm sinus of Valsalva				Both ventricles				
H	37	15672	M	66	Longshoreman	700	Left ventricle	Syphilitic aneurysm			+***			+		
I	38	15603	M	72	Laborer	320	Coronary artery	Subepicardial hemorrhage		L. coronary A.			+			
	39	376 GSH	M	57	Waterworks	600	Subepicardial capillaries	Old aneurysm		A.I.V.						
	40	24577	M	72	Cigar dealer		Coronary artery	Subepicardial hemorrhage	A.I.V.	A.I.V.			+			

Autopsy: The pericardial sac is distended with a dark blood clot. The heart weighs 380 gm. On the left surface of the left ventricle there is a ragged perforation 1 cm. in length through which the ventricle has discharged its blood. The adjacent myocardium is of a dull yellowish brown color and has a fatty appearance, and the wall in this region is thinned by old cicatrization. Areas of fibrosis are present throughout the left ventricle. The coronary orifices are patent, the right being very small. The coronary arteries are in general extremely narrowed by sclerosis and calcification. The anterior interventricular and left marginal branches of the left coronary artery, also the right circumflex and posterior interventricular branches of the right coronary artery appear to be occluded by sclerosis or organized thrombi. The aorta shows considerable arteriosclerosis with calcification.

Histology: The microscopic picture is that of a recent infarct. The anterior descending branch of the left coronary artery is closed by what appears to be sclerotic thickening of its wall. The posterior interventricular branch of the right coronary artery is closed except for several minute recanalizations. The microscopic picture of this artery is suggestive of syphilitic arteritis. The marginal branch of the left coronary artery contains a fresh thrombus. In spite of the strong suspicion of syphilis in these arteries, infarction has evidently resulted from fresh thrombosis of the left circumflex artery.

Pathological Diagnoses: Very recent infarct with rupture in the presence of fresh thrombus of marginal branch of left coronary artery; organization and recanalization of several other coronary arterial branches due to syphilis or to old healed thrombi.

CASE 28: (Path. No. 11,716). M. W., female, obese, aged 65, a Christian scientist, who had been subject to fainting spells, died without medical attention and came to autopsy at direction of the coroner.

Autopsy: The pericardial sac is filled with clotted blood that encloses the heart. The apex of the left ventricle is adherent to the parietal pericardium by fibrous bands and in this region is found an irregular ragged tear in the thinned bulging ventricular wall producing a free communication between the ventricular cavity and the pericardial sac. On incising this portion of the wall the myocardium is found to be replaced by a thin fibrous wall. The ascending aorta contains atherosclerotic plaques and the mouths of the coronary arteries are somewhat narrowed by sclerosis. The coronary arteries are sclerotic and the anterior descending branch of the left coronary artery is occluded by a grayish thrombus. There is a dissecting aneurysm of the descending aorta. The gallbladder has a thickened wall and contains multiple cholesterin calculi.

Histology: Microscopic examination confirms the gross finding of recent infarction implanted upon an old aneurysm of the left ventricular apex. Rupture has occurred at this site. Quite compatible with this combination of old and recent infarction is the microscopic demonstration of an old recanalized thrombus of the anterior descending branch of the left coronary artery and a fresh thrombus farther along in the course of the vessel.

Pathological Diagnoses: Aneurysm of apex of left ventricle with superimposed fresh infarct and rupture at the same site; both recent and recanalized thrombi of the anterior descending branch of the left coronary artery; chronic cholecystitis with cholelithiasis.

CASE 29: (Path. No. 13,543). E. B., male, fairly obese, aged 56, came to autopsy in the coroner's morgue following sudden death.

Anatomical Diagnoses: Recent anemic infarct and preëxisting old aneurysm of anterior wall of left ventricle with rupture; hemopericardium; arteriosclerosis of coronary arteries with calcification; fresh thrombus of anterior descending branch of left coronary artery, verified histologically; thrombosis of left ventricle; fibrinous pericarditis; anemic infarct of spleen; beginning gangrene of right foot; old amputation of left leg; chronic cholecystitis with cholelithiasis.

Comment: In this group as well as in Group A fresh coronary thrombosis was constantly found in the branch supplying the site of rupture. In addition, however, old organized and recanalized thrombi of coronary arteries were found, accounting for the old cardiac aneurysm present. The patients in this group usually have a previous history of coronary disease.

Group C. Recent Infarcts Due to Coronary Embolism (2 Examples)

CASE 30: (Path. No. 10,160). J. B., male, aged 76, a night watchman, died suddenly after breakfast and following physical exertion. He had previously been working and had not complained of illness.

Autopsy: On opening the pericardial sac the heart is found enclosed in a large blood clot. The posterior-lateral wall of the left ventricle near the apex is perforated by a ragged tear 2 cm. in length. The surrounding myocardium is hemorrhagic. The coronary orifices are freely patent. The coronary arteries are moderately sclerotic. The circumflex branch of the left coronary artery is apparently occluded by a reddish thrombus.

Histology: Microscopic examination discloses what appears to be a septic infarct. The left marginal artery contains an old recanalized thrombus. Branches of both the right and left coronary arteries contain thrombi that are probably embolic in origin. The source of these was not determined at autopsy.

Pathological Diagnoses: Septic infarct of the left ventricle with rupture in the presence of multiple thrombotic coronary emboli; recanalized left marginal artery.

CASE 31: (Path. No. 13,414). J. M. L., male, age about 70, came to autopsy in the coroner's morgue. No history was obtained.

Autopsy: The pericardial sac contains a large clot that encloses the heart. Two perforations are found in the wall of the heart. One on the posterior-lateral surface of the left ventricle is 12 mm. in length and is surrounded by several irregular reddish areas in the myocardium. The second, situated in the anterior wall of the right ventricle, measures 1 cm. in length. Atherosclerosis of the aorta is present in moderate degree. The coronary arteries are narrowed by sclerosis and calcification, and the right artery is apparently occluded by a thrombus. Sectioning of the myocardium discloses what appears to be an infarct of the left ventricle. The gall-bladder contains multiple calculi.

Histology: Microscopic examination discloses what appears to be infarction of the myocardium. The right coronary artery contains an old recanalized thrombus. In another portion of the same artery are embolic masses which appear to be loosened plaques from the arterial wall.

Pathological Diagnoses: Simultaneous rupture of right and left ventricles by separate openings; old recanalized thrombosis of the right coronary artery; embolism by loosened atheromatous plaques in another portion of the same artery; cholelithiasis.

Comment: The 2 examples in this group constitute sudden unforewarned deaths due to heart rupture following coronary embolism. In Case 30 the emboli are thrombotic and the infarct appears septic; in Case 31 the embolus consists apparently of material that had become dislodged from the lining of an artery and carried in the blood stream, in the manner described by Panum and others. This is presented in detail in a general review by one of us.[91]

Group D. Old Infarct and Aneurysm (1 Example)

CASE 32: (Path. No. 6093). J. A., male, aged 87, a seaman, had been hospitalized over a period of 5 months preceding death. His complaint had been shortness of breath, choking sensations and difficulty in swallowing, such that at times he had had to hold a spoon in his throat in order to facilitate deglutition. The temperature during the course of his illness had generally been subnormal, the pulse usually 80 to 90 and missing an occasional beat. The leukocyte count taken 2 weeks preceding death was 10,700. The manner of exitus was not observed.

Autopsy: The pericardial sac is distended with fluid and clotted blood. The heart, apparently of about 500 gm., is asymmetrical, the left ventricle being most prominent and roughly egg-shaped. There is a hernial bulging at the apex 4 cm. in diameter, slightly spherical, deep red in color and containing near its right margin a ragged opening that admits a 2 mm. probe. On the posterior aspect of the left ventricle 2 cm. from the first prominence is another rounded elevation which, however, is firm and unruptured. The second prominence proves on opening the heart to be an old infarct with areas of fresh hemorrhage, and from it a hemorrhage has dissected toward the thin apex, where external rupture has occurred into the pericardial sac. Diffuse fibrous pericarditis is present over the whole apical portion of the left ventricle. The coronary orifices are patent. There is considerable arteriosclerosis of the aorta and the coronary arteries are generally narrowed. The anterior descending branch of the left coronary artery appears to be occluded by an organized thrombus.

Histology: The microscopic picture is that of 2 old infarcts, 1 of them with aneurysmal thinning of the ventricular wall. A dissecting hemorrhage from the ventricular lumen has penetrated the old infarct in the left posterior wall of the left ventricle and has dissected to the apex causing rupture at that point. The anterior descending branch of the left coronary artery contains an organized thrombus with multiple recanalizations. No fresh thrombus is found.

Pathological Diagnoses: Recanalized thrombus of anterior descending branch of left coronary artery; multiple (2) old infarcts of left ventricle, 1 of them with aneurysm formation; dissecting hemorrhage through these infarcts with rupture.

Comment: In this type, as would be expected, the symptoms have extended over a period of months and have been characterized by

anginal attacks. Death has finally occurred from dissecting rupture of the chronic myocardial lesions. No fresh coronary thrombosis is present and an old recanalized thrombus accounts for the chronic myocardial lesions found.

Group E. Perforation of Interventricular Septum (2 Examples)

CASE 33: (Path. No. 10,923). O. C. B., male, aged 60, was found dead in bed in the morning. He had been ailing for some time but had not been confined to bed. He had sat up playing cards the night preceding death.

Autopsy: On opening the pericardial sac it is found to contain a little straw-colored fluid. The heart, which weighs 450 gm., is discolored posteriorly by a diffuse hemorrhage involving the interventricular septum and the posterior surface of the right ventricular wall. The pericardium is adherent at this point by fibrous bands. When the left chambers are opened a ragged rupture 2 cm. in length is seen in the muscular interventricular septum and connecting the right and left ventricles (Fig. 1). The perforation is directly below the discoloration in the posterior wall of the heart and has apparently occurred through a recent infarct.

The aortic lining is studded with yellowish plaques of sclerosis. The coronary orifices are freely patent, there being two right coronary ostia. The main branches of the left coronary artery are greatly narrowed by sclerosis and calcification. The left circumflex is occluded by an old whitish recanalized thrombus. The right coronary artery soon becomes occluded by a recent reddish thrombus extending over a distance of 2.5 cm.

On careful dissection of this heart a large, recent, mixed anemic and hemorrhagic infarct is observed in the posterior wall of the left ventricle extending into the septum, then for a short distance in the posterior wall of the right ventricle. The perforation of the septum is 4 cm. in length at its left opening and 3 cm. at its right, and extends through the septal portion of this infarct.

Histology: Microscopic examination discloses a recent infarct which, however, appears septic in type. The right coronary artery contains a fresh thrombus, evidently accounting for the large infarct in the posterior wall of the heart. The left coronary artery contains multiple recanalizations. Both of the arteries and the pericardium present a histological picture strongly suggesting syphilis, yet perforation of the septum in this case must be ascribed to infarction resulting from thrombosis of the right coronary artery in the presence of construction of the left coronary artery due to an old recanalized thrombus.

Pathological Diagnoses: Septic infarct of interventricular septum and the posterior wall of both ventricles with perforation of the muscular septum; fresh thrombosis of the right coronary artery; recanalized thrombus of the left coronary artery; probably syphilitic arteritis and pericarditis.

CASE 34: (Path. No. 12,529). A. J., male, aged 81, died the second day after admission into Multnomah County Hospital in a stuporous condition. His sister states that he had suffered a cerebral hemorrhage one year previously, with resulting auditory and speech aphasia but no paralysis. He had complained of pain in the upper abdomen and right chest for the past 3 years, but had otherwise enjoyed fair health.

Eight days before admission the family noticed that he was pale and short of breath. On attempting to climb the stairs he was forced to sit down on the first step, and was put to bed.

Examination showed left border of heart 13 cm. from the midsternal line. The apex beat was diffuse, and the point of maximum intensity in the sixth interspace. A loud systolic murmur was heard best over the mitral area and transmitted to axilla and back. The liver was tender to palpation and 3–4 fingers' breadth below the costal margin. The temperature was subnormal, pulse 100 and weak, respiration 30 per minute, blood pressure 140/70. Red blood corpuscles 3,000,000, hemoglobin (Sahli) 80 per cent, leukocytes 11,900. The blood Wassermann was negative. Four hours before death the patient's condition became critical — the skin cold and moist, the fingernails cyanosed, the pulse imperceptible.

Clinical Diagnoses: Motor aphasia; chronic myocarditis with mitral regurgitation and acute dilatation of heart.

Autopsy: The pericardium, both parietal and visceral, is rough, red, granular and covered with fibrinous exudate, but the pericardial sac contains no free blood. All chambers of the heart are dilated. The apex of the left ventricle is thin-walled. In the distal half of the muscular septum is a ragged tear about 1 cm. in length which perforates the septum and provides a communication between the ventricles. On sectioning the septum extensive myofibrosis is found but acute infarction is not grossly evident. The lining of the perforation appears somewhat old, as if it might have existed for some time. The anterior descending branch of the left coronary artery is occluded by a recent reddish thrombus. An old area of softening 3 or 4 cm. in diameter is found in the cortex of the left cerebral hemisphere.

Histology: Microscopic examination confirms the gross diagnosis of perforation which has existed long enough to allow the formation of granulation tissue. The anterior descending branch of the left coronary artery contains a fairly recent thrombus with canalization.

Cultural Results: Culturing of the affected area of the heart in blood agar gave a pure culture of non-hemolytic streptococcus.

Pathological Diagnoses: Fairly recent perforation of muscular interventricular septum through an infarcted area in the presence of thrombus of anterior descending branch of the left coronary artery with beginning organization.

Comment: Cases of rupture of the septum occur rarely, there being about 26 in the literature, and most of these are said to have been in the membranous septum. It is of interest that both of ours are in the muscular septum.

Both are due to coronary thrombosis with infarction. In Case 33 the arteries appear syphilitic, yet syphilis cannot be considered the direct cause of rupture, as coronary thrombosis and infarction were undoubtedly present. Of interest in this connection is Pember and Stangl's [92] report of interventricular septum aneurysm without rupture in a syphilitic subject. They attribute their aneurysm also to an old recanalized coronary thrombus rather than to syphilis.

Special interest attaches to Case 34, which was hospitalized. In this instance culture of the cardiac infarct and of the coronary thrombus yielded a pure culture of *Streptococcus viridans* and the possibility of sepsis suggests itself.

Group F. Saccular Aneurysm of Left Ventricle with Rupture (1 Example)

CASE 35: (Path. No. 10,725). W. E. J., male, aged 45, a lieutenant in the fire department, died suddenly while driving his car. Three months prior to death he had suffered from pain in the stomach radiating to the left arm, for which he consulted a physician. At that time he looked ill and suffered intensely. The heart was irregular and the pulse feeble. The blood pressure was 160 systolic. On the day of death he was on duty and feeling unusually well.

Autopsy: The pericardial sac is filled with clotted blood. Over the anterior surface of the left ventricle the pericardium and epicardium are adherent by firm fibrous tissue. On the upper antero-lateral surface of the left ventricle is a pouch-like sac about 4 by 4 by 3 cm. in diameter, whose walls are formed by the parietal and visceral pericardia and scar tissue. This pouch communicates with the cavity of the left ventricle by a narrow smooth-walled sinus only 5 by 3 mm. in diameter. The lining of this aneurysmal sac is whitish, smooth, except for radiating linear wrinkles extending outward in all directions from the opening, and continuous with attenuated trabeculae carnae and the endocardium of the left ventricle. For approximately 2.5 cm. about the opening through the ventricular wall the myocardium is the seat of patchy whitish scarring which is not present elsewhere in the heart. Like the ventricle the aneurysm contains fragments of blood clot. In the base of its inferior wall is a tear 4 mm. in length which dissects through the fibrous adhesions at the base of the pouch, finally rupturing into the pericardial sac midway between the pouch and the ventricular apex. All coronary arteries are narrowed by sclerosis and the smaller of two anterior descending branches of the left coronary artery passing toward the area of myofibrosis appears to be occluded by a white thrombus. No fresh thrombi are found. The ostium of the right coronary artery is apparently closed by sclerosis, while that of the left is greatly narrowed. The aorta, while slightly sclerotic, does not present the gross appearance usually associated with syphilis.

Histology: Microscopic examination discloses latent and healed lesions of the medial and adventitial coats of the aorta which are more compatible with rheumatic disease than syphilis. Vascularized atheromatous plaques are present in the coronary artery supplying the zone of scarring about the aneurysmal ostium, thus accounting for the gross appearance of a recanalized thrombus. The original lumen is reduced to almost microscopic size by the atheromatous process. The bulk of fibrous tissue forming the myocardial scar is obviously old, while a certain proportion of that composing the aneurysm wall gives evidence of more recent formation.

Pathological Diagnoses: Arteriosclerotic narrowing of coronary arteries; patchy myofibrosis of left ventricle with formation of saccular aneurysm, spontaneous rupture and fatal hemopericardium; focal fibrous pericarditis; atherosclerosis and probable rheumatic lesions of aorta.

Comment: The above example of heart rupture has certain points in common with Case 32, Group D, in which a dissecting hemorrhage occurred through the wall of a healed cardiac infarct. However, this case must be segregated from all other groups for the following reasons: (1) The lack of demonstrable coronary thrombosis; (2) the patchy character of the myocardial scar at the seat of aneurysm formation, more consistent with loss of muscle substance from slow ischemia as a result of arteriosclerosis than thrombosis with infarction; and (3) the unique character of the aneurysm, communicating with the ventricular cavity by a small opening.

Lack of space forbids a more detailed consideration of this form of aneurysm and we are publishing an account of it separately.*

Group G. Cardiac Rupture Due to Dissecting Aneurysm of a Sinus of Valsalva (1 Example)

CASE 36: (Path. No. 112 GSH). Mrs. D. D. W., a teacher, aged 50, died in Good Samaritan Hospital. Since childhood the patient had had chronic heart ailment. She was seen in an attack of sciatic rheumatism 4 months previous to death, and at that time looked sallow and complained of weakness, although still teaching school. Three weeks previous to death she complained of extreme weakness and had a chill, with temperature rising to 104°F. She gradually failed in strength and was admitted to the hospital, where she followed a septic course. A blood culture 24 hours before death yielded a pure culture of *Streptococcus viridans*.

Anatomical Diagnoses: Subacute bacterial endocarditis of *Streptococcus viridans* originating in a bicuspid aortic valve which had resulted probably from recurrent endocarditis; dissecting hemorrhage of right sinus of Valsalva extending upward into aortic wall, separating media and adventitia, also extending along interatrial septum and anterior wall of right auricle and ventricle, bulging into right auricle and ventricle, also bulging anteriorly into pericardial sac, with final epicardial rupture and fatal hematopericardium (Fig. 2); fibrinous pericarditis; extreme fatty infiltration of epicardium.

Comment: Dissecting aneurysms with rupture form too large a subject for full consideration here and for a more complete discussion the reader is referred to a previous review by one of us.[91] We have had another case, however, which is similar to the foregoing example in most respects but failed to rupture. A brief report of this might be of value here.

(Path. No. 7280). P. M., single, male, a gardener, with rheumatic history and with complaint of weakness, chronic cough, difficult breathing and shortness of

* Hunter, Warren C., and Benson, R. L. Saccular cardiac aneurysm with rupture. *Am. J. Path.* (in press).

breath, died in Multnomah County Hospital. Autopsy revealed: fibrous obliteration of pericardial sac; concentric hypertrophy and dilatation of heart (weight 760 gm.); saccular aneurysm of ascending portion of aortic arch, one pouch of which involves the left posterior sinus of Valsalva, and another pouch of which dissects into the interatrial wall and bulges into the lumen of the left auricle; chronic fibrous endocarditis of left auricle; senile arteriosclerosis and syphilitic aortitis; stellate scars and miliary gummas in arachnoid over cerebral sulci.

This example differs from the preceding in that it apparently constitutes a dissecting aneurysm arising in a syphilitic aneurysm of the aorta.

Group H. Rupture of Syphilitic Cardiac Aneurysm (1 Example)

CASE 37: (Path. No. 15,672). T. P. C., male, single, aged 66, a patient in the U. S. Veterans' Hospital, Portland, Oregon, had previously been a longshoreman and ship watchman. Illness began over 2 months before death and simulated pneumonia. Patient entered the Veterans' Hospital 11 days prior to death complaining of weakness, shortness of breath and with pain around heart extending up into left shoulder. He reported that his trouble began 2 months previously with choking which awoke him from sleep and that his lungs had been "tight" ever since.

Physical Examination: Generally negative. X-ray showed enlargement of the heart in all diameters. Wassermann test was positive. A week previous to death he was troubled with palpitation and dyspnea. Examination showed temperature of 98.3, pulse rate 78 recumbent and 88 standing, blood pressure 148/90, respiration 18, no heart murmurs or arrhythmias. Blood examination showed 7300 white cells, of which 64 per cent were polymorphonuclears. For several days previous to death he felt fairly well. At 7.30 of his terminal day he refused breakfast and complained of shortness of breath. The heart rapidly failed and in 15 minutes he expired.

Autopsy: The body is well developed and 180 cm. in length. There is no evidence of a scar on the penis but the inguinal lymph nodes are palpable. The pericardial sac is adherent to the anterior surface of the heart and also to the diaphragm. When the pericardial sac is opened it is found to be filled with clotted blood. The parietal pericardium is adherent over the whole anterior aspect of the apex and on removing it the friable and greatly thinned ventricular wall comes with it, exposing a dark mass of blood clot and necrotic tissue which distends the bulging apex of the ventricle. The aorta is atheromatous and sclerotic throughout its course and shows evidence of scarring with linear wrinkling. Both coronary ostia are patent. The walls of the coronary arteries are thickened by sclerosis but not to any extent calcified. Several of the anterior and marginal descending branches of the left coronary artery are nearly occluded by grayish thickening of the walls. Coronal sectioning of the left ventricle in the region of the aneurysm and rupture discloses extensive necrosis throughout the wall of the anterior half of the apex. The myocardium is mottled, containing yellowish areas mixed with grayish, semitranslucent streaks of fibrosis and containing extensive areas of hemorrhage (Figs. 3A and 3B).

Histology: Microscopic examination of the aorta discloses irregular fibrosis of the adventitia with slight perivascular lymphoid infiltration about some of the vasa vasorum. The adjacent pericardium is more densely infiltrated with similar cells and plasma cells. The media and intima show a slight fibrous increase with beginning hyaline degeneration. There is perivascular lymphoid infiltration about a few of the vasa vasorum in the media. The microscopic picture in the coronary arteries is especially interesting and distinctive. The adventitia is densely infiltrated with lymphoid cells and plasma cells. The media is irregularly thickened by sclerosis, accompanied by considerable hyaline degeneration and with considerable distortion of the wall. In places the lumen is almost completely occluded by layers of hyaline connective tissue. In places there is dense infiltration of the media by lymphoid cells and fibroblasts and this sometimes extends through the intima to the lining of the vessel. Atheromata and calcification are almost lacking. In the smaller arteries the walls are usually moderately to greatly thickened with fibrosis of the media. Microscopic examination of the myocardium in the region of rupture shows complete necrosis usually without fibrous tissue replacement. All traces of cellular structure are lost. The margin of this large area of necrosis is peculiarly free from extensive cellular infiltration, there being only a scanty infiltration of lymphoid cells. The myocardium in other portions of the heart wall contains irregular fibrous patches of small to large size which separate the strands of thinned and atrophied muscle fibers. Perivascular lymphoid infiltration appears in the interstitial tissue in places but is not a prominent feature.

Pathological Diagnoses: Syphilis of coronary arteries with extensive syphilitic aneurysm of left ventricular apex; rupture of this aneurysm into old pericardial adhesions with fatal hematopericardium; syphilitic myocarditis, pericarditis and mild syphilitic aortitis.

Group I. Rupture of Coronary Artery into Pericardial Sac (3 Examples)

CASE 38: (Path. No. 15,603). A. C., male, aged 72, died suddenly and reached the coroner's morgue.

Autopsy: The pericardial sac is found to be full of dark fluid and clotted blood. At the extreme apex of the left ventricle there is a diffuse blackish discoloration under the visceral pericardium. In the center of this area the surface is torn, producing a ragged slit 3 cm. in length. Sectioning shows that this rupture extends only into the subepicardial fat and not into the cavity of the ventricle (Fig. 4). The dark discoloration surrounding this is due to extravasation of blood. The ventricular wall is 2 to 2.5 cm. in thickness, except at the apex where it is only 1 cm. thick. The postero-lateral wall contains patchy areas of fibrosis but no evidence of infarction is found anywhere. Both coronary orifices are freely patent, the right being small. The ascending aorta is atheromatous but without any appearance suggesting syphilis. Both coronary arteries and their branches are considerably narrowed by atherosclerosis but without gross evidence of calcification. A branch of the left circumflex artery is occluded by an old white thrombus.

Histology: Microscopic examination of the aorta shows considerable hyaline degeneration throughout the media without any other noteworthy change. The walls of the coronary arteries are considerably thickened by fibrous increase with hyaline change in all coats. One branch of the left circumflex is almost occluded

by hyaline fibrous tissue and in the center are several small lumina. No occluding thrombi are found. Microscopic examination of the myocardium discloses a few areas of fibrosis but no evidence of infarction or any definite evidence of syphilis.

Pathological Diagnoses: Marked atherosclerosis of coronary arteries with narrowing; occlusion of branch of left circumflex artery by old recanalized thrombus. Rupture of branch of anterior interventricular coronary artery into subepicardial tissue at left ventricular apex with fatal hemorrhage into pericardial sac; no perforation of ventricular wall discoverable; patchy myofibrosis of left ventricle; atherosclerosis of aorta.

CASE 39: (Path. No. 376 GSH). W. D. C., male, aged 57, died in Good Samaritan Hospital. The patient had enjoyed fair health up to the time of a sudden attack of pain in the chest with dyspnea 4 months prior to death. Dyspnea had been severe during the few weeks preceding death. Physical examination showed ascites, also fluid at the bases of the lungs. The skin displayed a slightly icteric tint. Blood pressure registered 112/80.

The last four weeks before death were spent in the hospital, where the temperature had been constantly subnormal except during the last few days, when it fluctuated. The pulse had been mostly 100 or above, the respiratory rate usually over 40. The Wassermann was negative; white blood cells 14,800 4 weeks before death; red blood cells 5,000,000; hemoglobin (Sahli) 85 per cent. X-ray showed the heart "enlarged 50 per cent."

Autopsy: On opening the pericardial sac fibrous adhesions are encountered between the pericardium and epicardium over most of the anterior surface of the heart, dividing the sac into two parts. In these adhesions, and extending into the pericardial space to the left is a thin layer of freshly clotted blood (Fig. 5). Anteriorly the left ventricle bulges outward. The wall here is only 5–6 mm. in thickness, of cartilaginous consistence and of a pearly white color. The endocardium is greatly thickened and whitish. An organizing thrombus tampons the apex of the chamber. Cross-sections at frequent intervals fail to show a rupture of the heart wall at any point and all evidence points to rupture of capillary vessels in the pericardial adhesions as the source of the hematopericardium. There is pronounced arteriosclerotic narrowing of all coronary arteries with old recanalized thrombi nearly occluding the anterior descending ramus of the left coronary artery and two of its larger branches proximal to the old infarction.

Histology: Microscopic examination confirms the presence of recanalized thrombi in the arteries mentioned above. There is no evidence of syphilis. Capillaries and thin-walled blood sinuses of the epicardium in the region of the adhesions referred to above are found enormously distended with blood.

CASE 40: (Path. No. 24,577). W. K., male, aged 72, collapsed and died suddenly in the office of a physician whom he had consulted previously for irregularity of the heart rate following periodical drinking bouts. The blood pressure was 130/90. Nothing in the history indicated coronary disease. Just prior to death he complained of the heat, although the day was not hot, and was perspiring freely. Collapse occurred while conversing with the physician.

Autopsy: The pericardial sac contains about 100 cc. of blood which appears to have come from a small area of subepicardial hemorrhage at the apex of the left ventricle. There is no evidence of rupture although the muscle of the anterior wall of the left ventricle is soft and reddish. The lumina of the larger coro-

nary arteries are considerably narrowed by arteriosclerosis and that of the left anterior interventricular artery is occluded 2.5 cm. from its origin by a fresh thrombus situated distal to an older and recanalized thrombus. Unfortunately the heart was lost before a more detailed study could be made.

Histology: Microsections of the anterior descending branch of the left coronary artery disclose advanced atherosclerosis of the intima with great narrowing of the lumen and occlusion by a thrombus showing early organization at the periphery. In sections from the apex of the ventricle there is considerable fresh hemorrhage in the subepicardial fat but the source is not apparent. The *arteriae telae adiposae* are for the most part devoid of blood. The myocardial cells are fragmented but there is no evidence of infarction or of syphilis.

Pathological Diagnoses: Advanced atherosclerosis of coronary arteries with obstruction and occlusion of the left anterior interventricular vessel by recent thrombus showing early organization and by a recanalized thrombus proximally; probable rupture of this artery with subepicardial hemorrhage dissecting through epicardium to pericardial space; hemopericardium; atherosclerosis of aorta.

Comment: We have already given references to the literature on coronary arteriorrhexis. These are our only cases in which the fatal hemopericardium was due to escape of blood into the pericardial sac from coronary arteries. The reader will recall, however, that 2 others, Cases 4 and 12, suffered rupture of coronary arteries (multiple in Case 12) in addition to cardiac infarction. In all of our 4 cases of coronary artery rupture thrombosis of an artery seems to have been the causative factor, with the exception of Case 39, in which tearing of pericardial adhesions caused capillary hemorrhage.

Discussion

The more important related facts in this extensive series of cases can best be collected in the form of a table (Table I). The clinical history, whenever available, has been given briefly in connection with each individual case report, and no further comment is necessary here.

Our series of cases present a diversity of location. Two of them represent perforations of the interventricular septum. Another constitutes an intrapericardial rupture of a dissecting aneurysm of a sinus of Valsalva. Case 39 appears to be a fatal hemorrhage into the pericardial sac from tearing of adhesions over an old aneurysm of the left ventricle. Cases 38 and 40 consist of a diffuse subepicardial hemorrhage from rupture of a coronary arterial branch with fatal leakage into the pericardial sac, the original cause apparently lying in an old organized thrombosis of a coronary branch (Case 38) and

in a recently formed thrombus in the other (Case 40). Two other examples of coronary arteriorrhexis, Cases 4 and 12, occurred in the presence of fresh coronary thrombosis and cardiac infarction. In the first of these, rupture of the heart wall resulted with discharge of the ventricular blood into the pericardial sac, while in Case 12, thrombosis of a coronary branch was followed by dissecting hemorrhage in the wall of the vessel, which tore its way through the myocardium and epicardium with fatal hematopericardium.

Of the 34 cases representing rupture through the whole thickness of the left ventricular wall, 11 occurred anteriorly, 6 posteriorly, 9 laterally at the *margo obtusus*, and 7 at the apex. Two of the posterior ruptures deserve special mention. One (Case 24) began near the junction of the left ventricle and muscular septum and tore through the epicardium near the *margo acutis* after having dissected through the right posterior ventricular wall for several centimeters. Another (Case 31) posterior left ventricular perforation was accompanied by a second rupture in the anterior wall of the right ventricle.

The importance of Case 35 has already been referred to in connection with the case history, in that it constitutes the only known example of its kind. A sacculated aneurysm formed and developed a smooth lining, but finally, possibly after a long period of time, ruptured by a dissecting hemorrhage, first into pericardial adhesions and then into the pericardial sac.

Relations of age, sex, and occupation can best be obtained from Table I. Physical exertion appears to have been commonly an immediate forerunner of ruptures, as mentioned in the individual case reports.

An attempt has been made to establish definitely in each instance the condition of the coronary arteries and the consequent rôle of arterial thrombosis or embolism in distinction to syphilis or other specific infectious causes. This has necessitated careful coronal sectioning of each artery in the previously fixed heart, and has in nearly every instance been confirmed by microscopic examination of the supposed thrombus. After exhaustive histological study of the arteries and of the myocardium we feel reasonably assured that our criterion of infarction is correct in each case.

In our 25 examples of rupture of a recent infarct which have been grouped together under Class A, we have demonstrated the throm-

botic occlusion of the coronary artery in all except the heart in Case 11, which was lost before examination was completed. Some of these hearts have indeed manifested microscopic evidence of syphilis of the coronary arteries, myocardium, and aorta, as noted in the table, but even in these instances unquestioned thrombosis of the coronary vessels has been proved.

Likewise, in the 2 infarct examples, Cases 30 and 31, the immediate cause has been demonstrated in the form of emboli. Again in the 4 cases of Group B, those having a recent infarct on the basis of a preëxisting cardiac aneurysm, the recent arterial thrombus has been found, usually along with evidence of an earlier organized thrombus of coronary arteries. In another heart, Case 32, an old infarct had ruptured, and, as might be expected, an old organized thrombus of a coronary artery was found. In the two interventricular perforations, Cases 33 and 34, we have demonstrated coronary thrombosis as the forerunner of the cardiomalacia. The probable genesis and atypical character of the aneurysm in Case 35 render this case so unique that it is being described in a separate paper.* In the 3 remaining instances of coronary arteriorrhexis, Cases 38, 39 and 40, we have likewise demonstrated old organized coronary thromboses. Thus, 38 of our series of 40 appear to be examples of cardiorrhexis attributable to recent or old coronary occlusion by thrombi, emboli or atherosclerosis.

The 2 cases falling outside of this coronary occlusion group are 36 and 37, and of these Case 36 obviously represents the dissection of a mycotic aneurysm of the aortic valve due to *Streptococcus viridans*. The remaining specimen (Case 37) appears syphilitic, and the reader is referred to the detailed record for evidence to that effect. This subject gave a positive Wassermann and the heart and aorta bore typical evidences of syphilis. It is reasonable to assume that in this instance syphilis was probably responsible for the rupture.

We have already mentioned that several other hearts in the series showed microscopic evidences of syphilis in addition to the more certain presence of coronary thrombosis. We cannot admit, however, that syphilis is responsible for the cardiorrhexis unless indeed it can be allowed that this infection is a common precursor of coronary thrombosis. Such a relationship has never been established.

* Hunter, Warren C., and Benson, R. L. Saccular cardiac aneurysm with rupture. *Am. J. Path.* (in press).

Finally, attention is called to the high incidence, 17.5 per cent, of cholelithiasis in our series.

Summary

Forty ruptures of the heart have been collected from nearly 7000 autopsies in Portland, Oregon. One of these was probably of syphilitic origin. Another, a dissecting aneurysm of a sinus of Valsalva, was due to *Streptococcus viridans* endocarditis. The remaining 38 ruptures, although in some instances manifesting evidence of syphilis, were attributable to recent or old thrombosis, embolism or arteriosclerosis of the coronary arteries.

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DESCRIPTION OF PLATES

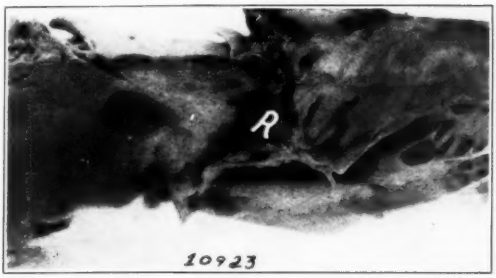
PLATE 51

FIG. 1. Case 33. Sectional view of septum. Natural size.
R = Site of rupture.

FIG. 2. Case 36. Intrapericardial rupture of dissecting hemorrhage from sinus of Valsalva due to *Streptococcus viridans* endocarditis. Right ventricle opened.
A = Aortic valve.
H = Dissecting hemorrhage.

FIG. 3 A. Case 37. Coronal section through site of rupture.
R = Site of rupture.
N = Necrotic area.
T = Intraventricular thrombus.
A = Wall of aneurysm.

FIG. 3 B. Case 37. Coronal section proximal to rupture.
H = Dissecting hemorrhage.
N = Necrotic area.
T = Intraventricular thrombus.



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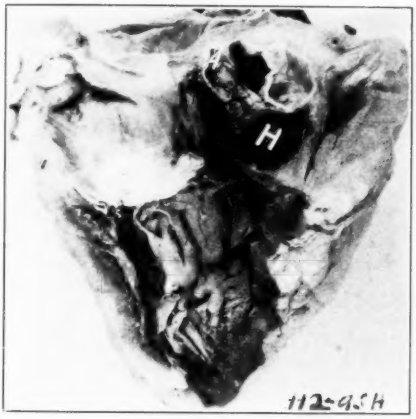


2

3A 3B

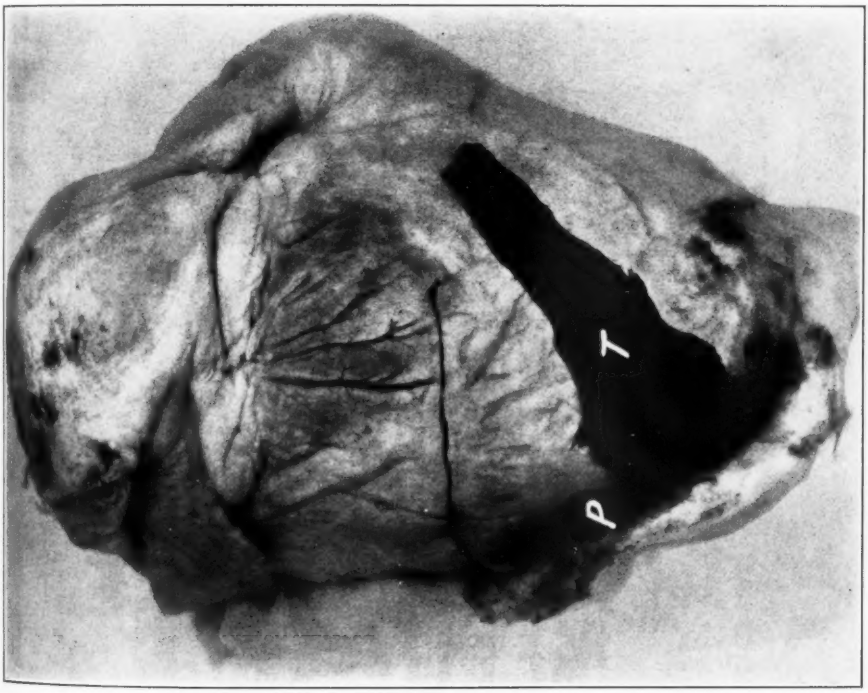
Benson, Hunter, Manlove Spontaneous Rupture of Heart

PLATE 52

FIG. 4. Case 38. Rupture of coronary branches with resulting subepicardial hemorrhage (H) and final rupture into pericardial sac.

FIG. 5. Case 39. Hematopericardium due to tearing of pericardial adhesions overlying old healed aneurysm of left ventricle.
P = Parietal pericardium, reflected back, showing torn adhesions.
T = Portion of intrapericardial thrombus.

4



5

A COMPARISON OF THE CELLULAR REACTION IN EXPERIMENTAL TUBERCULOSIS OF THE CORNEA IN ANIMALS OF VARYING RESISTANCE *

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Previous experiments by Vorwald [1] have shown that a definite correlation does not exist between the intensity of cellular reaction in the lung in different animals after implantation of tubercle bacilli, and the subsequent development of tuberculosis in this organ. His studies at the same time indicated that a large percentage, perhaps the majority, of the cells taking part in the formation of the developing tubercles under the conditions of his experiment came from the blood stream. A study by Long, Vorwald and Donaldson [2] on the early cellular reaction to tubercle bacilli in the testis in normal and tuberculous guinea pigs and guinea pigs immunized with dead bacilli, led to the same conclusions.

Vorwald's observations brought out again with unusual clarity the facts in the course of tubercle development first described by Borrel [3] and his associates, and recently emphasized by Albert-Weil,[4] *viz.*, the immediate influx of polymorphonuclear leukocytes at the point of lodgement of tubercle bacilli and the subsequent phagocytosis and replacement of these cells by large mononuclear cells. The apparent vascular origin of most of the cells concerned suggested the value of a similar study of tubercle formation in different animals in the non-vascular cornea. It seemed possible that differences in the rate of tubercle growth in different animals might be still more conspicuous in the latter organ.

Four series of animals were inoculated with human type tubercle bacilli (H 37 of Saranac Lake), *viz.*, normal and tuberculous guinea pigs, rabbits and cats. Interlamellar injections were made with sharp, short beveled, fine gauge hypodermic needles in or near the center of the cornea in each case. It was impossible to fix the dosage exactly because of the minuteness of the amount of fluid that could be injected into the cornea without detrimental trauma or uncon-

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trolled spreading, but the approximate amounts of tubercle bacilli injected were 0.01 mg. in the rabbits and cats, and 0.005 mg. in the guinea pigs. A sudden, sharply limited, white opacity 2–3 mm. wide in the cornea in the region inoculated, on slight pressure of the loaded syringe after the needle was in place, signalized the success of the injection.

The course of development of the infection in the different animals is summarized in Figure 1. The relative sizes of the lesions are shown by fine shading at the site of infection, and blood vessels are indicated by small circles, at the limbus in all animals and at the site of infection in the sensitized guinea pig killed at 2 weeks and the normal guinea pig and rabbit killed at 1 month. Approximate simple differential counts are given of the cells partaking in the reaction, the region at which the count was made being indicated by an arrow in each case.

At 6 hours no reaction was apparent in any animal except at the limbus. After the passage of 24 hours a cellular reaction was seen in the injected area, in or bordering on the center of the cornea, in every animal. In the figure attention is drawn to the limbus reaction at the early and to the central reaction at the later periods.

Significant facts from observation of sections, including those stained for tubercle bacilli, are as follows, and the following conclusions seem warranted in their explanation.

1. The first cellular response apparent in the infected cornea was around the blood vessels of the limbus in every instance; it was notable here 6 to 24 hours before a reaction could be detected at the actual site of injection. This seemed good evidence that the cells responding immediately to the infection came from the blood vessels at the margin of the cornea, and not from the conjunctival secretion along the needle track, as was claimed by many of the early observers.

2. A considerable variation in the limbus response was seen in the different animals of the series at the 6 hour period. The most intense reaction occurred in the sensitized (tuberculous) guinea pig. The severity of reaction in the normal guinea pig, rabbit and cat decreased in the order named. The prompt outpouring of cells in the already infected guinea pig appeared simply another example of the well known hypersensitivity in this type of animal. It could quite appropriately be considered a tuberculin reaction, and indeed other

experiments by ourselves have shown that the same divergence in intensity of reaction in the cornea occurs in normal and tuberculous guinea pigs following the intracorneal injection of purified tuberculin protein. The difference in intensity of initial reaction in the normal animals was similar to that observed by Vorwald in the lung, and is further evidence that intensity of reaction at the outset does not predicate corresponding speed of suppression of the infection. On the contrary, the least reaction was observed in the cat, the animal of the highest general resistance to infection.

3. The predominant cell in the limbus reaction at 6 hours, the earliest period studied, was the polymorphonuclear leukocyte in all instances. At 24 hours cells of this type had reached the point of inoculation in large numbers, whereas few mononuclears had yet appeared at this point. Large mononuclears were present at 24 hours in considerable number, however, in the zone of reaction at the limbus. As time went on, as shown by sections at later periods, mononuclears gradually increased in number in the central zone of reaction at the site of infection, and rapidly increased when blood vessels reached this point. These facts, taken together, seemed to warrant the conclusions that (*a*) both the polymorphonuclear leukocyte and the large mononuclear in the developing tuberculous lesion in the cornea come from the vascular system, and (*b*) the former reaches the infected region sooner because it is a more rapidly moving cell. The failure of large mononuclears in the early reaction at the site of infection seems good evidence that the local cells do not partake in the reaction, as claimed by Baumgarten [5] in his early work.

In passing it should be said that objection may be made to these conclusions because of the size of the dose of tubercle bacilli used in the experiments here recorded. Krause [6] has stressed the point of dosage in the following words: "If we eliminate all disturbing factors that may arise through the inoculation of too large quantities of bacilli we may arrive at a true appreciation of the character of the process which gives rise to the aggregation of epithelioid cells which form primary tubercle. In response to the localization of a very few bacilli and during the first few days after localization, we find that not a vestige of tissue disturbance occurs except at and around the immediate point of localization of bacilli. Between this site and any other point we cannot trace the slightest evidence (anatomical) that the bacilli have exerted the least effect on any other cells than those

of the immediate neighborhood. Save for the completely isolated aggregation of epithelioid cells, enclosing tubercle bacilli, the microscopic field discloses entirely normal tissue — of lung or cornea for instance. At the same time, within the epithelioid masses cells in active mitosis are frequently to be observed. These two phenomena compel the opinion that the *origin* of nodular tubercle, that is, the first aggregation of epithelioid cells, is accomplished through the *proliferation*, in other words the new growth, of cells at or near (within microscopic distance of) the point of localization of tubercle bacilli, and normally existing there."

The question of the origin of the cells entering into the reaction will be considered in detail, in connection with further experiments, in the next chapter.

In explanation of the size of the dose used in our experiments we should say that we desired to follow the fate of the tubercle bacilli injected, and for this reason injected a quantity making this effort easy. The amount injected is not excessive according to the usual standards for the inoculation of tubercle bacilli. Nevertheless, in order to answer the question of relation of type of reaction to dosage we diluted the suspension of bacilli used in the experiments here reported 100 times for an additional, otherwise similar experiment in the rabbit cornea. It is significant that although the intensity of reaction was very much reduced, qualitatively no difference was observed from that seen and here described with the larger dose. Accordingly we felt that valid conclusions for the general subject of tuberculous infection could be drawn from our more extensive experiments with the doses of 0.005 to 0.01 mg. of bacilli.

4. The tubercle bacilli injected were practically all phagocytozed by polymorphonuclear leukocytes within the first 24 hours. With the arrival of the large mononuclear phagocytic cells, ingestion of the polymorphonuclears by these cells occurred, with a corresponding transfer of the tubercle bacilli first taken up by polymorphonuclears. The situation was like that described and illustrated by Vorwald, except that in the present case the arrival of the large mononuclears was a much delayed process. At 72 hours the predominant cell at the site of infection in all animals was still the polymorphonuclear leukocyte, but at the two later periods studied, 2 weeks and 1 month, the large mononuclears were in considerable excess, and the bacilli were chiefly within their cytoplasm.

5. The final picture showed characteristic differences. The sensitized guinea pig was dropped from the series recorded in the accompanying figure, because at this period in reinfected guinea pigs ulceration occurred and heavy secondary infection complicated the picture. In the other animals the intensity of the reaction, as measured by the proportion of the cornea in a state of inflammatory reaction, varied in the guinea pig, rabbit and cat, with decrease in the order named. At 1 month vascularization of the infected area was well developed in the guinea pig and rabbit, and in these animals the cells of the lesion were now predominantly mononuclear. In two out of three cats, on the other hand, vascularization of the infected area had not yet occurred and the proportion of mononuclear cells of inflammation was much lower. As the cat had the highest general resistance to infection with the strain of tubercle bacillus used, and the guinea pig the least, the intensity of reaction at 1 month, the period of full tubercle development, was seen to be in inverse proportion to the general resistance.

Summary

Central interlamellar corneal injections of 0.01 to 0.005 mg. of moderately virulent tubercle bacilli of human type were made in a series of normal and tuberculous guinea pigs, and normal rabbits and cats. Animals of each kind were killed at 6 hours, 1, 3 and 14 days and 1 month, and the nature and extent of the inflammatory response determined by microscopic examination.

In all cases the first reaction noted was at the limbus, and not at the site of injection. The reaction consisted of an outpouring of polymorphonuclear leukocytes from the marginal vessels. It was most intense in the tuberculous guinea pig (tuberculin reaction), less in the normal guinea pig, still less in the rabbit and least in the cat.

The first cellular reaction at the site of injection in the center of the cornea consisted of leukocytes migrating in from the margin. The reaction was well developed in all animals at 24 hours, and the cells taking part were almost exclusively polymorphonuclear leukocytes.

After 24 hours there was a gradual increase in the proportion of large mononuclears in the reaction. The rate of increase rose with the closer approach of blood vessels, as vascularization of the cornea

developed. The paucity of these cells at the earlier periods and abundance in the vascularized state of the cornea seemed good evidence that the large mononuclear leukocyte in corneal tuberculosis is not locally derived, as formerly claimed. In all animals the large mononuclears, as they reached the site of infection, engulfed the polymorphonuclear leukocytes already there, taking over the tubercle bacilli contained by the latter cells.

From 3 days to 2 weeks the inflammatory reaction progressed most rapidly in the tuberculous guinea pigs, and less rapidly in the normal guinea pigs, rabbits and cats in the order named. Ulceration occurred in the tuberculous pigs in 2 weeks, and these pigs were dropped from the series at this point.

At 1 month, the last period studied, the intensity of inflammatory reaction, as determined by the size of the lesion and proportion of the cornea involved, was greatest in the guinea pig, less in the rabbit and least in the cat. This order is in inverse relation to the general resistance of these animals to the strain of tubercle bacillus used.

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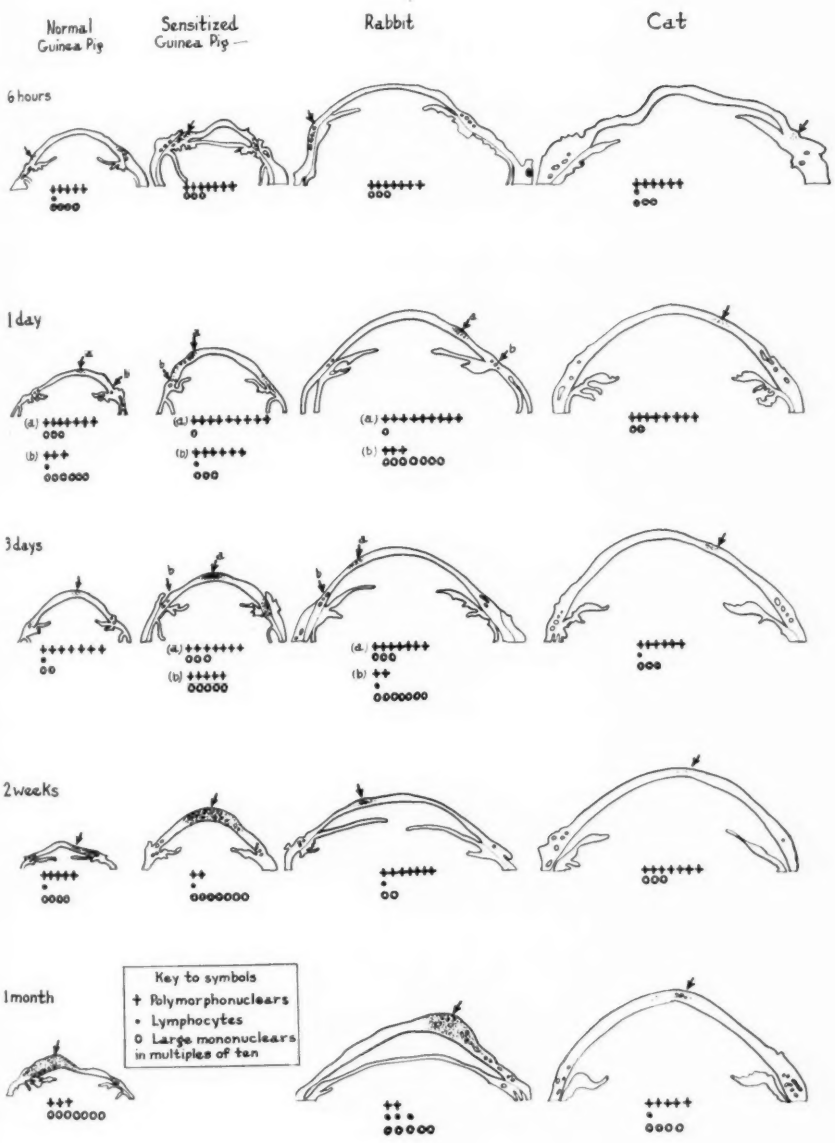
DESCRIPTION OF PLATE

PLATE 53

FIG. 1. Course of development of tuberculosis of the cornea in different animals. The size of the lesion is indicated by fine shading. Blood vessels are indicated by small circles and ovals. Approximate differential counts of the cells are given, the regions at which the counts were made being marked by arrows.


Normal Guinea Pig
Sensitized Guinea Pig
Rabbit
Cat
6 hours
1 day
3 days
2 weeks
1 month
Key to symbols
+ Polymorphonuclears
• Lymphocytes
0 Large mononuclears in multiples of ten

THE ORIGIN OF THE EPITHELIOID CELL IN EXPERIMENTAL TUBERCULOSIS OF THE CORNEA *

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The cornea has frequently been used in the experimental study of inflammation. Surrounded by capillaries, but normally avascular itself, it is an ideal site in which to study the movements and focalizations of wandering leukocytes. It is therefore not surprising that even fifty years ago it was employed in the attempt to determine the origin of the characterizing element of the tubercle, the so-called epithelioid cell. In 1885 Baumgarten [1] undertook a study that has formed the starting point for numerous subsequent investigations. Even at this early date there was dispute as to whether the characteristic cells of the tubercle were derived from the blood stream or from fixed tissue elements.

Baumgarten, using a procedure already fruitful in the hands of Cohnheim and Koch, planted bits of tuberculous tissue in the anterior chamber of rabbits' eyes and watched the course of development of tuberculosis in the adjoining structures. In a second series of animals he inoculated the cornea directly with suspensions of ground tuberculous organs. He distinguished two types of reaction, (1) an immediate acute one, marked by rapid influx of wandering cells; and (2) one occurring several days later around new foci of multiplying bacilli, characterized by mitoses among the fixed tissue cells, and subsequently by the appearance of cells of an epithelioid type. The first reaction he thought was due to trauma as well as to the bacilli injected, and he was of the opinion that the greater part of the cells entered from the conjunctival fluid. The second he looked upon as the characterizing response of tuberculosis. His conclusion was that the typical epithelioid cell of the tubercle in the cornea is a derivative of descendants of the fixed tissue cell, and not a wandering leukocyte. He held the same view in regard to the epithelioid cells in tuberculosis of other organs.

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Although not unchallenged, this conception remained dominant until recently. For a clear epitome of the Baumgarten view the reader is referred to Krause,[2] who adopted it, with certain reservations, in his exposition of the anatomical structure of tubercle from histogenesis to cavity. More recent studies in hematology and cytology, however, have thrown new light on the problem, and at the same time have shown it to be much less simple than was formerly supposed. Among these are the extended studies of Sabin and her coworkers on experimental and clinical tuberculosis, and experimental tubercles produced by the injection of purified substances from the tubercle bacillus. Using the supravital technique Sabin, Doan and Cunningham [3] traced the origin of the epithelioid cell to the monocyte, pointing out that the latter is a cell both of the connective tissues and of the circulating blood. The extensive studies of Maximow [4] and Bloom [5] on the transformations of lymphocytes and monocytes have shown that epithelioid cells may develop by a series of changes from stem cells, both of the blood stream and of local tissue. Although common ground can be found in the positions of the different investigators in the field, a good deal of confusion has developed as the result of attempts to make sharp distinctions on the basis of source and function between the various large mononuclear, potentially phagocytic cells. Recently Sabin [6] in reviewing the conflicting evidence on relationship between the monocytes and macrophages, as developed by herself and her colleagues, and by Maximow, Bloom, M. R. and W. H. Lewis, and Carrel and Ebeling, has stated the reconciling conception toward which there now seems a general trend. This is, briefly, that the monocyte and clasmatocyte are merely stages of the same cell, their differences depending on the material they have phagocytozed rather than on differences in cytoplasm adapting them to different functions. Her own studies have indicated that the typical monocyte of the connective tissues and blood is a younger type of cell than the typical clasmatocyte of the connective tissue. The recent experimental studies of Hetherington and Pierce [7] on the transformation of cells of the monocytic class into typical tissue macrophages and further into epithelioid and giant cells, bear out this view.

The cornea seems especially well suited to further investigation of some of the puzzling questions thus raised. Previous studies [8] have shown that injection of tubercle bacilli into the cornea of rabbits and

other animals is followed, as in other organs, by an accumulation first of polymorphonuclear neutrophile leukocytes around the bacilli, and then of large mononuclear wandering cells which engulf and replace the leukocytes. It seemed significant that arrival of the large mononuclear cells approximately coincided with the appearance of capillaries in the injured, previously avascular area. This observation made it seem probable that the large mononuclear cells, which ultimately gave rise to epithelioid cells, came either from the blood stream directly, or from the walls of the new ingrowing blood vessels, and not from local fixed tissue cells of the cornea.

In order to test this hypothesis an experiment was devised for comparing the development of tuberculous areas in the same eye, but respectively near and far from the original blood supply. It seemed probable that if the source of the epithelioid cells was the vascular system a region of infection near the limbus would become of epithelioid character more rapidly than one in the center of the cornea. Accordingly in each of a series of large rabbits two infections with 0.01 mg. human type tubercle bacilli (H 37), of low but still appreciable virulence for this animal, were introduced in the same cornea, one about 2 mm. from the limbus, the other in the center or 5 to 6 mm. from the limbus. Animals were killed at 1, 6, 14 and 48 days. The infected eyes were fixed in formol-Zenker's fluid, and the corneal half of each was embedded in celloidin. The blocks were mounted in such a way that the plane of sectioning included the site of both infections and the intervening tissue. Three sets of sections were prepared, one stained with hematoxylin and eosin, one with hematoxylin, carbol fuchsin and orange G, and one with eosin-azure. By the second technique the tubercle bacilli and the granules of the polymorphonuclear leukocytes were well shown. The third technique was used in attempting to differentiate the mononuclear leukocytes.

The experiment yielded the results anticipated. Representative fields from the two sites of infection at each period are shown in the accompanying figures.

At 1 day the cell responding to the infection was the polymorphonuclear neutrophile leukocyte. Cells of this type were much more numerous at the limbus, close to the blood supply, but the reaction at the center, although less intense, was qualitatively the same, and thin lines of leukocytes could be seen streaming from the

limbus between the connective tissue fibers, all the way to the center, where they had formed a small focus around the bacilli.

At 6 and 14 days the animals yielded mutually similar results. In each a significant difference was now apparent between the reactions near and remote from the limbus. Near the limbus the invading cells, which at 1 day were almost all polymorphonuclears, were now mostly large mononuclears. Occasionally migration figures of large mononuclear cells could be seen in the walls of the blood vessels (Fig. 3). New capillaries could be found in this region, larger and better formed close to the limbus, but not yet within 2 to 3 mm. of the other site of infection at the center. In the latter non-vascular region the reacting cells were still chiefly polymorphonuclear leukocytes, although a few lymphocytes and large mononuclears were to be seen. At intermediate points, where vascularization was more or less advanced, a mixture of the two cells was found.

At 48 days another significant change was apparent. The reaction in the outer zone, near the limbus, was still almost exclusively of mononuclear character, but the cells were of a more mature type, including large aggregations of typical epithelioid cells and many fibroblasts with associated collagenic fibers. The cornea in this region was now at least double its original thickness. Some spots of necrosis had developed. In the center the reaction, although less intense, was qualitatively similar to that near the limbus. Vascularization was well developed from the periphery to the center of the cornea. Polymorphonuclear leukocytes had practically disappeared and in their place there were large mononuclear wandering cells in varying stages of maturity.

Wherever the cellular response was characterized by the appearance of large mononuclears, *i. e.* near the limbus in the 6, 14 and 48 day rabbits, and at the center in the 48 day rabbit, these cells were present in greatest concentration around the blood vessels. From this observation alone it could not be determined whether they were derived from the lumen or the wall of the vessel, but several other features of the reaction threw further light on this question. The characteristic arrangement of cells in the newly developed tissue is shown in Figure 7. Typical and atypical vessels could be distinguished. Commonly, as shown in more detail in Figures 5 and 6, the new vessels were surrounded by a collar of large mononuclear cells. In hematoxylin-eosin and eosin-azure sections the nuclei were seen

to be rich in chromatin, and spherical or oval, with only a slightly longer axis in one direction, or, more rarely, irregular with a slight tendency to bilobate form. The cytoplasm was small in amount, but somewhat more extensive than in normal lymphocytes, and basophilic. Cells similar to these were also found mixed in with red cells within the lumina of these vessels. Although the total number of such cells within the vessels was small as compared with the number outside, it was in great excess of the normal, and migration figures derived from such cells could be seen in the vessel walls.

In Table I are given counts of cells in and around cross-sections of three new capillaries. As these counts are representative of the scores of young capillaries present, no more need be given. It is apparent that the ratio of mononuclears to erythrocytes is much higher than in normal blood.

TABLE I

Cell Counts In and Around Cross-Sections of Three New Capillaries

Cells in the vessel lumen			Cells in and around the vessel wall
Erythrocytes	Mononuclears	Polymorphonuclears	Mononuclears
37	8	1	41
19	2	1	10 (one typical migration figure)
56	6	1	79 (including several apparently passing through the endothelium)

Atypical vessels filled with large mononuclears similar to those in the perivascular collars just described, were scattered throughout the new inflammatory tissue, as shown in Figure 7. One is shown in detail in Figure 5. In some instances these were lined by a typical layer of endothelium; in others a thin eosinophilic fibrillar membrane without nuclei formed the limiting wall. In other cases cells of identical character, free of endothelial or other limiting membrane, lay in sharply circumscribed masses, circular or oval in cross-section, within tissue spaces. On examination of serial sections many of these masses of cells could be traced back to the perivascular collars. All of these structures appeared to be simply developing branches

from the new capillaries, equipped with endothelium close to the vessel, and without it at a distance from the vessel. At some distance the large mononuclears seemed to be simply pushed along into tissue spaces.

Outside of the perivascular collars of chromatin-rich, large mononuclear cells, transitional forms of these cells could be recognized. These may be seen in Figures 5, 6 and 7. The nuclei were in general character similar to those seen in the perivascular collars and in the extensions from them described above, but certain important changes were apparent. The nuclei were larger and the chromatin was more widely dispersed. In the perivascular collars the chromatin granules were large and close together. In the cells a small distance away the chromatin granules were of similar size but more widely separated. The cytoplasm was less basophilic. A considerable variation in size and degree of change from the perivascular cells could be distinguished. Still further away from the new vessels a rather abrupt change took place to a type of cell with a larger, pale nucleus of less granular character and an eosinophilic cytoplasm. In the zone of transformation mitoses could be seen, but only rarely. The extreme degree of change was to a cell of typical epithelioid character. Sharply circumscribed areas of degeneration and necrosis with pyknosis and karyorrhexis occurred within masses of such epithelioid cells. In other words characteristic tubercles had formed. Only giant cells were lacking to make the typical picture complete.

In brief summary the process of chronic inflammatory reaction in the cornea seemed to be as follows.

1. Vascularization of the infected region.
2. Appearance of primitive mononuclear cells in (*a*) blood vessel lumina and (*b*) blood vessel walls.
3. Appearance of primitive mononuclear cells in perivascular arrangement.
4. Transformation through several stages to similar cells radiating from the perivascular accumulations.
5. Further transformation to typical epithelioid cells undergoing degeneration and necrosis in circumscribed areas.

The new tissue thus appeared to be made up of new blood vessels

and the perivascular mononuclear cells and their derivatives. The key to understanding of the histogenesis of the new inflammatory tissue seemed to lie in the conspicuous perivascular cellular collars, as these were evidently the source of all the new tissue outside of all the blood vessels.

Three observations seemed significant in determining the origin of these perivascular cells. First, similar cells could be seen in the vascular lumina, as shown in Figure 14. Second, similar cells could be seen occasionally caught by the process of fixation in the act of migration through the vessel walls. Several of these are shown in Figures 9–13. The number of such migration figures was not large, but probably represented only a small fraction of the migrations actually taking place at the time of the fixation. Experimental support for this view is found in the effect of small amounts of fixing fluid on the migration figures of tissue culture. The admixture of minute drops of Zenker's fluid with a growing culture causes prompt rounding up of moving cells with contraction of all their pseudopodia (unpublished observations of Aronson). In the sections under consideration here many mononuclear cells were found adherent to the walls of the capillaries, which might have been in the process of migration when the warm material was transferred from the animal body to the fixing fluid. The third observation of importance was the rarity of mitotic figures in the dense perivascular cellular collars. Occasional mitoses were indeed observed, but they were less than one-tenth as numerous as the migration figures.

All these facts taken together led us to believe that the chief source of the mononuclears surrounding the young capillaries was the blood stream, rather than the fixed tissue cells of the vessel wall. The mitoses rarely observed around the capillary walls were perhaps evidence that the fixed cells of this site participated to some extent in the formation of the perivascular collars. They seemed, however, a much less frequent source than the blood stream.

It has not seemed advisable in this paper to attempt to designate the important cells considered other than as primitive mononuclear cells. The interpretation of hematoxylin-eosin and eosin-azure stains varies with different observers, and supravital staining, if not subject to the same criticism, was impossible here because of the necessity of preserving topographical relations. The relationship of these cells to primitive lymphocytes and monocytes is thus left unsolved.

They appeared to be entirely different from the typical clasmatocyte, which has a more abundant cytoplasm. Cells of the latter class also were observed in small numbers in the perivascular collars, and the mitoses found occasionally among the cells outside of the collars appeared traceable to them. The cells forming the greater part of the perivascular mass were smaller and characterized by much denser nuclear stain. Their transformation to cells of an epithelioid character appeared to come about through progressive increase in size and change in character of the original cells, without the intermediary process of mitosis. This sort of change has been described by Maximow [9] as a "rapid hypertrophy" in his exposition of the transformation of the monocytic cells of the blood stream into large phagocytes. The cells seen in the massive chronic inflammatory reaction here described appeared to attain their ultimate epithelioid character gradually, not only without mitotic division but also without the formation of distinctive intermediary cell types.

Summary

1. Infection of the center of the rabbit cornea with tubercle bacilli of human type resulted in a lesion characterized almost solely by polymorphonuclear leukocytes until the arrival of new capillaries, when a rapid replacement of the polymorphonuclears by mononuclear phagocytic cells occurred. When two infecting doses were placed in the same cornea, one at the margin near the normal blood supply and one in the normally avascular center, the replacement of polymorphonuclears by large mononuclears occurred weeks earlier in the lesion close to the limbus.

2. The mononuclears accounting for this replacement and functioning in the subsequent development of the lesion apparently arose from primitive smaller mononuclear cells present in large numbers in and around the walls of the new capillaries. The source of these cells could not be determined with absolute certainty, but the following observations were evidence that most of them came from the blood stream: (*a*) cells of similar character were present among the erythrocytes in blood vessel lumina in great excess of the normal proportion; (*b*) migration figures of similar cells could be seen in the walls of the growing capillaries, and (*c*) mitotic figures among them were rare.

3. The primitive cells after accumulating in and around the capillary walls underwent a progressive transformation by simultaneous increase in size and change of character, without mitosis, into epithelioid cells.

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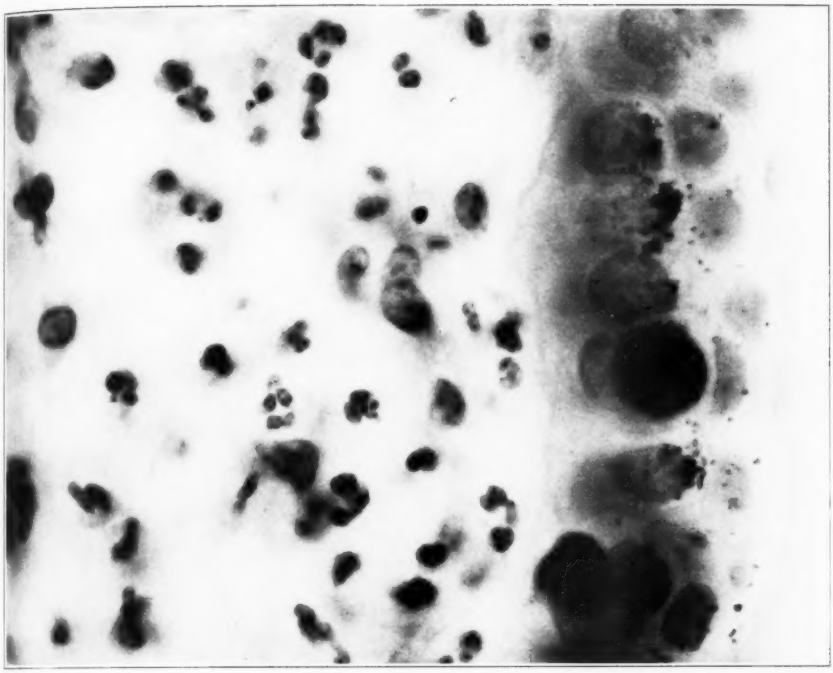
DESCRIPTION OF PLATES

PLATE 54

FIG. 1. Cornea of rabbit 24 hours after double injection of tubercle bacilli. Section from site of injection at margin. Note preponderance of polymorphonuclear leukocytes in inflammatory reaction. × 1200.

FIG. 2. Cornea of same rabbit 24 hours after double injection of tubercle bacilli. Section from site of injection at center. Note preponderance of polymorphonuclear leukocytes, which in this section are heavily stained for the granules. × 1200.



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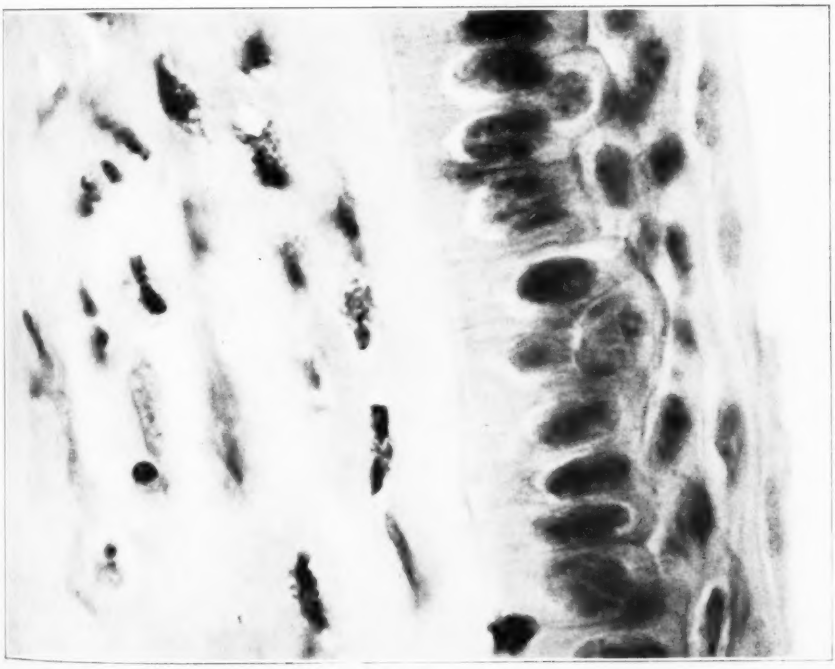


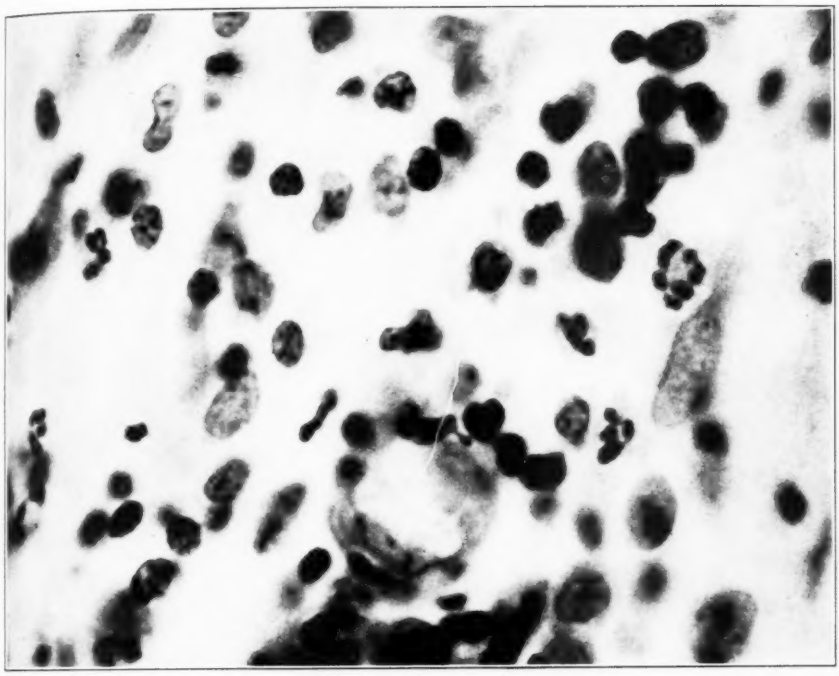
2

Long and Holley Epithelioid Cell in Experimental Tuberculosis

PLATE 55

FIG. 3. Cornea of rabbit 2 weeks after double injection of tubercle bacilli. Section from site of injection at margin. Note the new blood vessel and the preponderance of large mononuclear leukocytes in the inflammatory reaction. × 1200.

FIG. 4. Cornea of same rabbit 2 weeks after double injection of tubercle bacilli. Section from site of injection at center. Note persisting preponderance of polymorphonuclear leukocytes and absence of blood vessels. × 1200.



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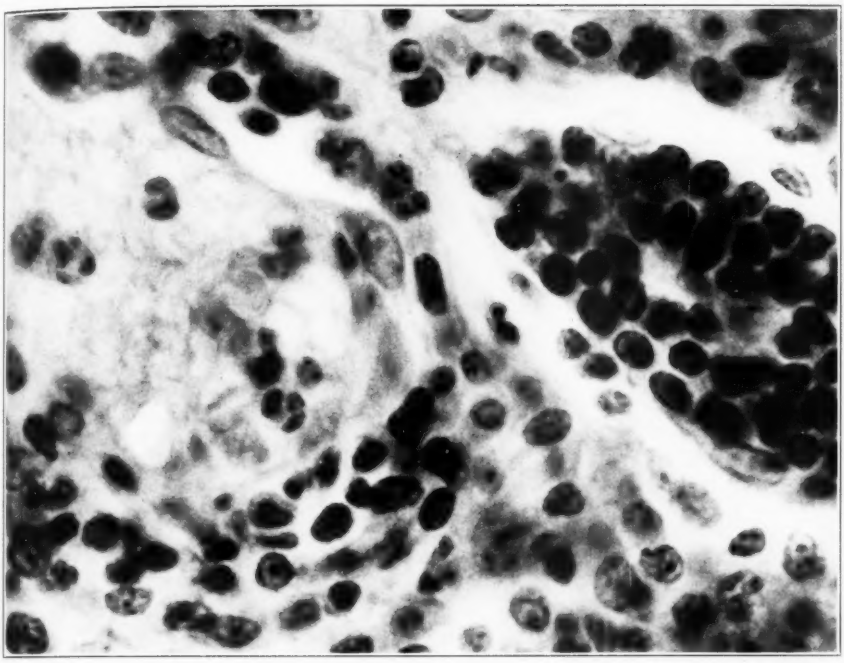
4

Long and Holley Epithelioid Cell in Experimental Tuberculosis

PLATE 56

FIG. 5. Cornea of rabbit 48 days after double injection of tubercle bacilli. Section from site of injection at margin. Note new blood vessels and accumulations of large mononuclear leukocytes around and in new vessels. Note also gradual change in character of large mononuclears outside of perivascular collar of cells. × 1200.

FIG. 6. Cornea of same rabbit 48 days after double injection of tubercle bacilli. Section from site of injection at center. Note new blood vessel and collar of hyperchromatic large mononuclear leukocytes. Very few polymorphonuclear leukocytes remain. Note changing character of large mononuclears outside of perivascular collar of cells. × 1200.



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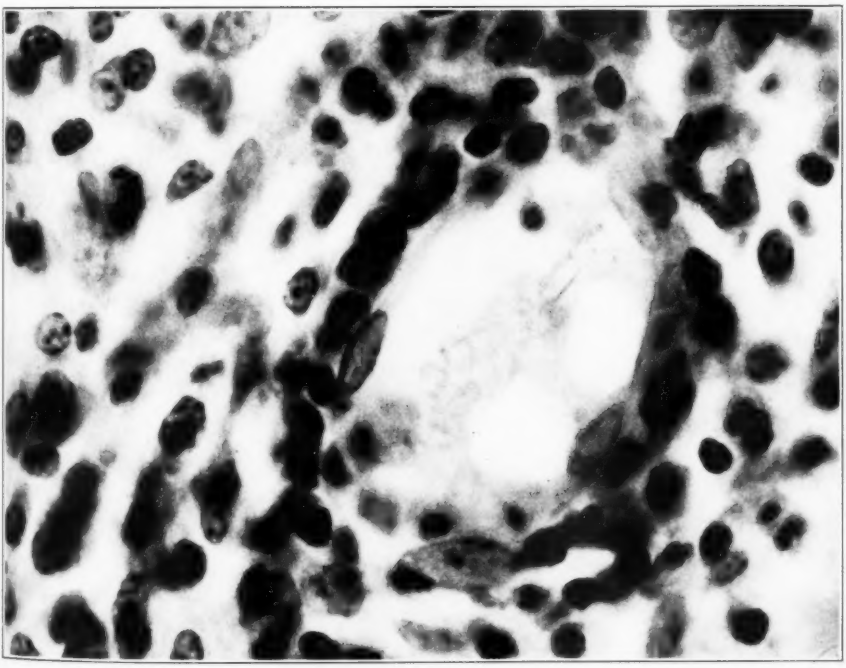

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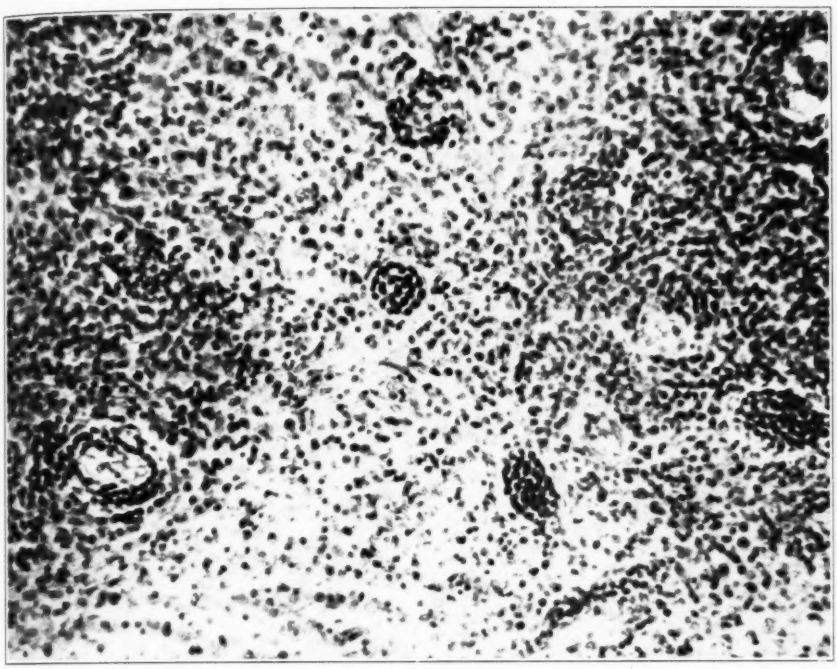
Long and Holley Epithelioid Cell in Experimental Tuberculosis

PLATE 57

FIG. 7. Cornea of rabbit 48 days after double injection of tubercle bacilli. Section from site of injection at margin. Note accumulations of large mononuclear leukocytes in and around new blood vessels and in tissue spaces. Note change from dense hyperchromatic nuclei in and near vessels to vesicular nuclei in surrounding tissue. × 260.

FIG. 8. Cornea of same rabbit 48 days after double injection of tubercle bacilli. This section also from site of injection at margin. Note new blood vessels and cells with hyperchromatic nuclei in association with them and note spreading of these cells away from vessel walls into surrounding tissue. Note change of cells to epithelioid type and region of necrosis (typical tubercle). × 50.



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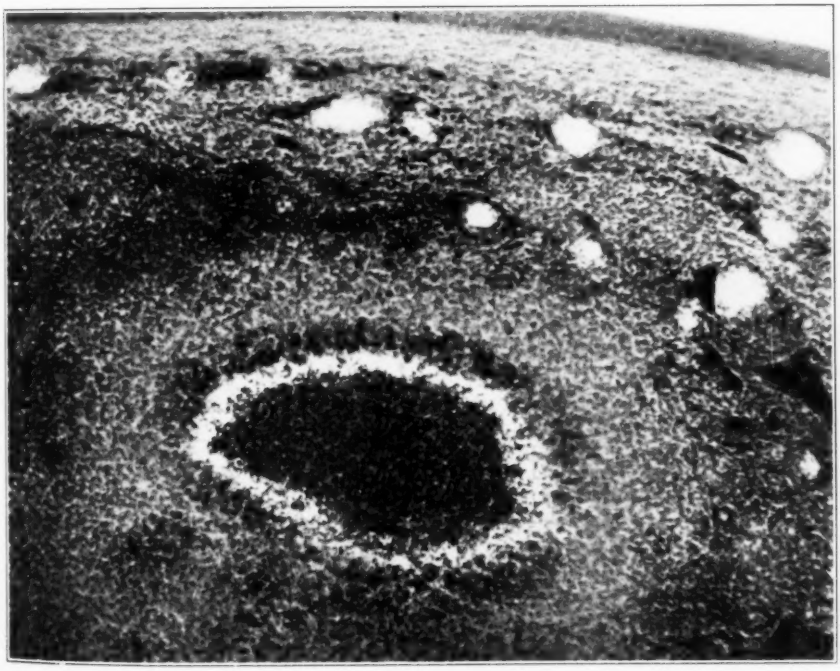


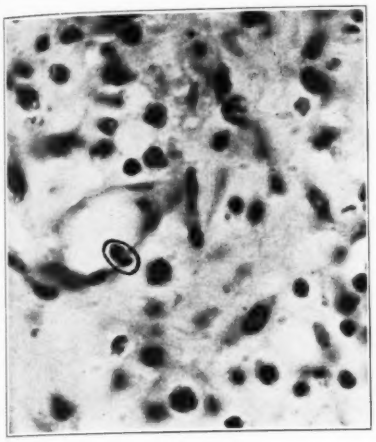
8

Long and Holley Epithelioid Cell in Experimental Tuberculosis

PLATE 58

FIGS. 9–13. New blood vessels in rabbit cornea 1 month after injection of tubercle bacilli. Note migration figures (outlined) of large mononuclear leukocytes in course of transit from inside to outside of vessel. × 600.

FIG. 14. New blood vessel in same rabbit cornea (outlined). Note large number of large mononuclear leukocytes in lumen. × 600.



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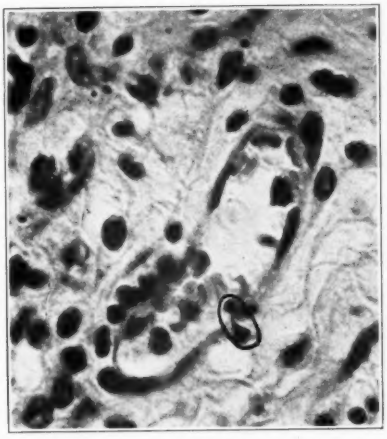

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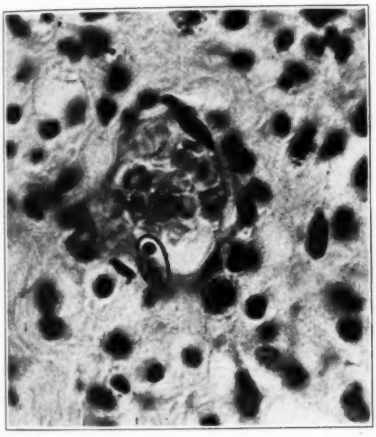

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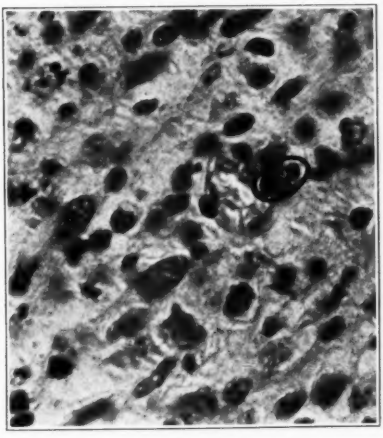

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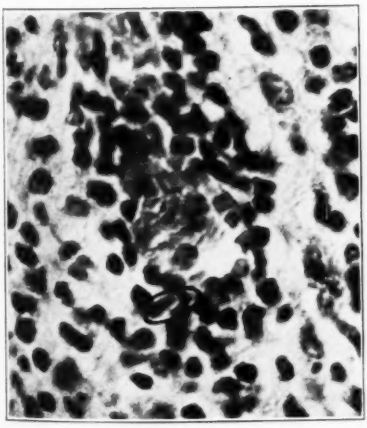

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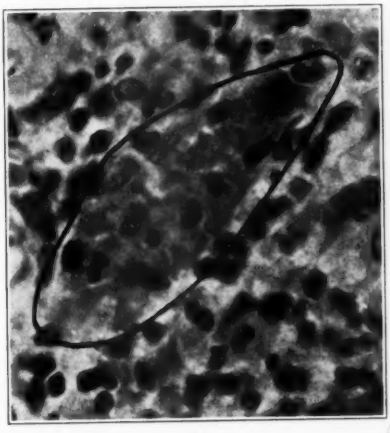

14

Long and Holley

Epithelioid Cell in Experimental Tuberculosis

THE HISTOLOGICAL CHANGES IN THE THYROID GLAND OF THE WHITE RAT EXPOSED TO COLD*

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In a series of articles beginning in 1916 and ending in 1928 Cramer and Ludford,[1-4] working in London, recorded in rats and mice exposed to cold increased vascularity of the thyroid gland, cellular hypertrophy with enlargement of mitochondria and Golgi apparatus, altered staining properties of the colloid followed by a decline in its amount and, eventually, death of the epithelium. This was interpreted as meaning increased secretory activity of the gland induced by the metabolic strain. Conversely, at warm temperatures the colloid increased in amount, the cells became flatter and the injection diminished. The importance of these observations lies (1) in the rôle of the thyroid in heat regulation thus suggested, (2) in their bearing on the long known seasonal incidence of endemic goiter, and (3) in the promise that they give of providing us with a means of modifying thyroid structure by an easily manipulated experimental condition that can safely be regarded as demanding, through the normal channels, augmented secretion. It is chiefly with this last interest in mind that the present study has been directed toward the resolution of the discrepancies existing between the work of Cramer and certain of his successors, discrepancies which have their ever perplexing counterparts wherever the effect of stimulated metabolism on the histology of the thyroid has been sought.

In Cramer's published reports experimental details are given sketchily, so that it is often difficult to get an exact picture of the environment and of the degree and time of histological change. Some effects were observed in "a mouse exposed to cold for three hours," "a mouse kept four hours in a laboratory with an open window during cool weather in March," and "a rat epilated and kept in a glass jar on a cold winter day." More extreme changes are figured in 2 rats kept in a cold room at 4° C for 10 and 14 days respectively. Ludford [4] obtained the most extreme changes in rats epilated and

* Received for publication January 13, 1933.

kept 24 hours in an ice-chest. The effect of heat (30–35° C) was apparently visible in 6 hours in the mouse.

Mills,[5] working in South Dakota in 1918, found quite regularly, as compared to the preliminary biopsy, increased cell height, vacuolation of the colloid and a decline in its amount in 16 rabbits and 4 guinea pigs exposed in a cold room or outdoors at −5 to +10° C for from 3 to 30 days. In 28 rabbits, 2 cats and 3 guinea pigs exposed in a well ventilated hot-box at 27 to 37° C all but 4 animals showed decreased height of epithelium and increase in the amount of non-vacuolated colloid.

Hart,[6] in Berlin in 1922, reported on gray house mice kept at 32 to 40° C until they died and at 4 to 7° for a time not stated. The pictures given for high temperatures are for 1 animal kept 38 days and another "several days." The epithelium is cubical, the colloid reduced, the follicles folded and empty. At low temperatures the cells are cubical, the colloid abundant. Verbally, Hart agrees with Cramer, Ludford and Mills that the thyroid is more "active" at low temperatures; actually, as Wegelin [7] points out, his findings are contrary to theirs as he uses accumulation instead of reduction of colloid to measure activity.

Bergfeld [8] in 1930, working in Freiburg, found that the thyroids of 3 male rats and 1 female, kept in the darkness in a cold cellar at from +2 to −3° C for 4 to 6 weeks, showed cellular hypertrophy and loss of colloid, while those of 3 males kept 6 weeks in a cellar illuminated by unfiltered daylight were normal. Again, in animals living 6 weeks in thermostats at 29° C, 3 in darkness had hypertrophy of the thyroid epithelium, while 3 in the light did not. Bergfeld concludes accordingly that darkening acts without reference to temperature to cause changes that indicate an "increased function," and that temperature alone is without influence. While the pictures of these Freiburg animals reveal differences too slight to convince, Bergfeld later,[9] working at Davos, Switzerland, adduced better evidence that the thyroids of rats deprived of ultraviolet light for 21 to 63 days were hypertrophied and poor in colloid. Rosenkranz [10] in Davos, in a small number of rabbits examined after 4 weeks, and Turner and Benedict [11,12] in New York, in a large number of Barred Rock chicks observed for from 55 to 105 days, confirmed Bergfeld's account of the effects of the absence of ultraviolet light, so that despite the negative findings of von Fellenberg [13] the factor of illumination must be taken

seriously. As neither Bergfeld nor Turner and his coworkers were able to detect any increase in the basal metabolism of the darkened animals, the mechanism remains for the present quite obscure.

NOTE: Space does not permit citation of the extensive scattered literature on the seasonal incidence of endemic goiter, nor of such studies of the geographical distribution of thyroid diseases as have a bearing on the effects of environmental temperature. Although of great interest, they are non-analytical in nature and in no case permit decision as to the relative importance of light, temperature and food supply. In the absence of any complete review of this subject the reader may refer to the articles in the monograph by Kendall.[14]

METHODS

Albino rats bred from a single stock were used. They were housed in quarters with a temperature range of 20 to 30° C, illuminated by diffuse daylight with some sunshine passing through windowglass to the cages. They were fed the standard Steenbock diet [15] consisting of:

Yellow corn	76.0
Linseed oil meal	16.0
Crude casein	5.0
Ground alfalfa	2.0
Sodium chloride	0.5
Sodium carbonate	0.5
Milk and water *ad lib.*	

Young animals from 43 to 105 days of age, weighing from 73 to 260 gm., paired for weight and sex and chosen in the great majority of cases from the same litter, were selected for experiment and as controls. Separate controls were run for each experimental period, as a single sampling of the stock does not suffice where temperature, light and food of necessity vary, and any or all may be of influence.

Most of the experiments were run in complete darkness. Exposure to cold was secured in a large, dark, refrigerated room in the hospital morgue, where the average temperature during the several experimental periods varied from -4 to $+2$° C. Ventilation, secured only by frequent openings of the door of the chamber, was sufficient to keep the oxygen and carbon dioxide content of the air, with animals present, not far from that of the ordinary dwelling room (O_2 — 20.76%, CO_2 — 0.1%). The occasional introduction of a corpse painted with iodine for surgery was unfortunately unavoidable, and although during several experimental periods this was known not to have occurred, the iodine content of the atmosphere must have been

high at times. While this may have contributed to the variability of the results it could not, as will appear subsequently, have materially altered the essential conclusions. The animals were placed 2 or 3 in a cage with beds of shavings and fed the stock diet with milk, or the following modification with water:

Yellow corn	62.0
Linseed oil meal	13.0
Crude casein	4.0
Ground alfalfa	2.0
Whole milk powder	18.0
Sodium chloride	0.5
Calcium carbonate	0.5
Water as desired	

The fact that the refrigeration of this chamber was controlled by a central plant operated to supply the entire building made it impossible to regulate the temperature locally. During certain experimental periods the fluids supplied the animals were observed not to be frozen at the time of feeding, while at other times freezing occurred and the chipping of the ice by the animals was insufficient to secure the usual intake. As the water consumption of a chilled rat is small and the chamber damp, no serious systemic effect of the restriction was likely, nor was any observed. The influence on iodine intake will be discussed later.

The controls were kept in a large, well ventilated photographer's dark room at average temperatures for the several periods of 20 to 31° C, fluctuating, of course, with the season. They were caged, cleaned, fed and watered precisely the same as their brothers and sisters in the cold.

At the completion of the run the animals were weighed and killed by a blow on the head. The thyroids were removed as rapidly as possible without sacrificing tissue and placed directly in Zenker's or formol-Zenker's fluid for fixation. Shortly thereafter the glands were weighed on an analytical balance, the difference between such weights and the fresh weights being insignificant. Under any circumstances the weights of the glands are to be regarded as approximate only, variations in drying and adherence of bits of surrounding muscle and fat precluding pretensions to refinement. The tissues were then dehydrated and embedded in paraffin. Sections were made serially through the entirety of both lobes of the first 110 glands

studied, and thereafter through the center of the larger lobe. Staining was with hematoxylin-eosin.

In interpreting slides arbitrary grades were assigned to the height of the epithelium, size and colloid content of the follicles, vacuolation of the colloid and vascular injection, without knowing the source of the tissue. Estimations were reached from serial studies of the entire thyroid mass when the sections were available, and later from central sections, which are, on the whole, representative. Vacuolation was recorded as absent or present in grades of 1, 2 and 3; injection as minimal (0) and as grades 1 and 2. These two estimations are difficult and are in addition subject to error introduced by variations in fixation and in vascular engorgement of the region at the time of death, and are hence to be regarded as the merest approximations. Cellular size was recorded as flat epithelium 1, cuboidal 2, low columnar 3, and high columnar 4. Amount of colloid was recorded as little or none, 0 – 1; small follicles filled with colloid, 2; medium follicles filled with colloid, 3; large follicles filled with colloid, 4. Occasional occurrence only was indicated by placing the figure in parenthesis. No attempt was made to assign any degree of follicular filling between that of completeness and that of practical depletion, in certain cases large plicated follicles that had obviously suffered a loss of content being passed as full. This error, which was infrequent, tends toward underestimation of the colloid loss. While such designations as these poorly depict the variety of living tissue, they are useful in reviewing data and in making tabular presentations of anatomical material. Moreover, with experience, they can be made with fair accuracy.

The Control Thyroid Gland

The thyroid glands of 64 control animals kept in the dark for from 5 to 25 days, together with those of 23 others receiving ordinary illumination, may be conveniently described together as the baseline for the present work (see the next section). The gland is a bilateral structure usually fairly symmetrical, but showing at times every grade of asymmetry, not infrequently to the absence of a lobe. The isthmus is usually only a connective tissue strand, but may be parenchymatous. The average weight is 12.7 mg. per 100 gm. body weight, varying from 4.1 to 25.4 with only 5 exceeding 20 mg. per 100 gm. It

may be so small as not to be discovered by the ordinary dissection. Microscopically the two lobes correspond. The epithelium is, as a rule, cuboidal to low columnar and the amount of colloid moderate (graded 2, 3 to 3) (Figs. 2 and 4). The follicles may be large or small throughout a gland, with a regular distribution of larger, well filled follicles lined with flatter epithelium lying superficially. Centrally the follicles are smaller, the cells a little higher, and foci of diminished colloid content, marked often by hypertrophy, appear in 17 per cent of the controls. While of varying size these foci are usually small, being only once large enough to dominate the gland. It is of interest that of the 15 control glands that have such areas of colloid reduction 11 occurred, for no known reason, in the first 32 studied during a period from March to May, 1931, the phenomenon being rare since. Adenomas have never been seen.

The Influence of Light

A brief preliminary comparison was made at 20° C between the effects of darkness and diffuse daylight entering the room through a glass window. Five pairs of litter-mates were kept for 10 days, one group in the dark room and the other in a well lighted laboratory. Table I, Part 1, shows that there was no difference between them. Table I, Part 2, includes these, and in addition 22 others of approximately the same age but not strict litter-mate controls, that were examined between March 11 and May 28, 1931. The height of the epithelium, degree of colloid reduction and gland weights were the same. Inspection of controls kept for periods of 15 to 25 days in the dark at 25° to 31°, as for instance those given in Table III, showed no tendency to reduction of colloid or hypertrophy of epithelium. Darkness alone, then, whatever its effect may be with stricter comparisons over longer periods of time, does not, within 25 days, produce striking hypertrophy and colloid loss. This is not intended to contravert the evidence concerning the importance of ultraviolet rays for thyroid function, but merely to emphasize that the striking effects to be detailed in animals exposed to cold cannot be attributed to exclusion of light.

The Effect of Exposure to Cold

A group of preliminary experiments of animals exposed to cold for from 6 to 24 hours showing nothing in particular, attention was directed to protracted experiments lasting from 10 to 25 days. Only such will be reported here.

The mortality was surprisingly low, especially if animals weighing over 80 gm. were used. A series of comparisons of 17 animals kept at

Table I

Comparison of the Thyroid Glands of Rats Kept 10 Days in Light and Darkness, March 11–May 28, 1931

Experiment	No. of animals	Amount of colloid									Height of epithelium					Average wt. of thyroid mg. / 100 gm.
		0–1			0–1, 2	2	2, 3	3	3, 4		3, 4	3	2, 3	2	1, 2	
		Most	foci	absent												
Part 1. Litter-Mate Controls																
Light at 20° C	5	0	1	4	0	0	1	4	0		1	2	2	0	0	10.2
Dark at 20° C	5	0	2	3	0	0	3	2	0		2	1	2	0	0	10.2
Part 2. Litter-Mate Controls Plus Others of Same Age																
Light at 20–21° C	12	0	4	8	0	3	2	7	0		2	5	5	0	0	12.9
Dark at 20–24° C	20	1	6	13	0	3	10	4	2		6	4	6	4	0	14.2

Key: The first series of columns under "amount of colloid" designates the occurrence of areas of little or no colloid (0–1) throughout the gland, in foci or absent. The second series of columns gives the distribution of various grades of follicular colloid content predominating in such glands as have more than traces: 2 = small follicles filled with colloid; 3 = medium follicles filled with colloid; 4 = large follicles filled with colloid. Such conjoint expressions as 0–1, 2 indicate that both degrees are found in abundance. Under "height of epithelium" 4 indicates high columnar; 3 = low columnar; 2 = cuboidal, and 1 = flat. Conjoint expressions as 3, 4 indicate that both types are present in large numbers.

0° and −3° C for 10 days with their controls showed no decline in the rectal temperature. The animals exposed to cold only rarely failed to gain weight, but such gains, as a rule, amounted to little more than half that of the controls.

Ensuring an abundance of food made little difference. Table II presents evidence that the amount of weight gained is in no way correlated with the degree of change described in the thyroid gland.

Age, within the limits studied, sex and stage of the oestrus cycle were without discernible influence on the structure of the gland.

Grossly the glands of the animals exposed to cold were often turgid, red, and at times massively enlarged. Of 72 rats exposed to temperatures averaging from 0 to $-4°$ for from 10 to 25 days in 8 series between March 1931 and March 1932, 32 showed reduction of colloid to traces through the greater bulk of the gland, 29 reduction in foci of various sizes, and in only 11 no such reaction. The epithelium was low to high columnar in 59, and in one case only was low cu-

TABLE II

Comparison of the Effects of Maximal and Minimal Weight Gain on the Thyroid Glands of Rats Exposed to Cold. Comparative Animals of Similar Age and Sex, Run at the Same Time, Are Selected from Groups in Table III

Experiment	No. of animals	Amount of colloid								Height of epithelium					Average wt. of thyroid mg. 100 gm.
		Most	9–1 foci	absent	0–1, 2	2	2, 3	3	3, 4	3, 4	3	2, 3	2	1, 2	
Maximal (average + 32.2 gm.)	13	7	6	0	4	2	2	0	0	13	0	0	0	0	19.7
Minimal (average + 7.4 gm.)	13	5	6	2	3	1	3	1	0	9	1	3	0	0	19.9

For key see Table I.

boidal to flat. Sixty-two of these animals had 49 strict controls, the great majority litter-mates (Table III). Of the experimental group 26 showed preponderating reduction of colloid to traces, of the control rats 1; 25 reduction in foci, of the control 5; no such reduction in 11, in the control 43. High columnar epithelium prevailed in 49 of the 62 experimental animals and in 5 of the 49 controls (Figs. 1, 3 and 5).

The average weight of the gland per 100 gm. body weight was 19 mg. in the experimental, 13 mg. in the controls. In 7 cases the weight of the gland in the experimental animals exceeded the maximum of the control glands, including such values as 62.5, 50.8 and 38.2 mg. per 100 gm. Out of 57 strict comparisons the gland of the animal exposed to cold was 1 mg. or more per 100 gm. body weight heavier than the control in 42.

TABLE III

Comparison of the Thyroid Glands of Animals Kept in the Cold with Those at Normal Room Temperature

Experiment	No. of animals	Amount of colloid									Height of epithelium					Average wt. of thyroid mg. 100 gm.
		0–1			0–1, 2	2	2, 3	3	3, 4		3, 4	3	2, 3	2	1, 2	
		Most	foci	absent												
Four Groups April 3 to May 29, 1931																
10 days 0 to −3° C	17	11	5	1	2	1	2	1	0		15	1	1	0	0	17.4
10 days 20 to 24° C	15	1	4	10	0	3	7	2	2		4	3	4	4	0	14.8
June 12 to July 2, 1931																
20 days −3° C	7	7	0	0	0	0	0	0	0		7	0	0	0	0	34.8
20 days 31° C	6	0	0	6	0	0	2	2	2		0	1	4	0	1	12.3
August 24 to September 18, 1931																
25 days −1° C	7	6	0	1	0	0	0	1	0		6	1	0	0	0	19.9
25 days 28° C	7	0	0	7	0	1	1	4	1		0	3	1	1	2	11.8
October 9 to November 2, 1931																
20 days −2° C	6	1	3	2	2	0	0	1	2		5	1	0	0	0	17.8
20 days 24° C	4	0	1	3	0	1	0	2	1		1	0	2	0	1	10.9
December 11 to 28, 1931																
17 days −3° C	13	1	7	5	2	4	5	1	0		6	2	3	1	1	16.5
17 days 25° C	7	0	0	7	0	0	7	0	0		0	0	2	5	0	12.3
February 19 to March 11, 1932 — Modified Diet with Water																
21 days −4° C	12	0	10	2	6	0	5	0	1		10	1	1	0	0	14.0
21 days 24° C	10	0	0	10	0	0	5	5	0		0	6	4	0	0	12.4
All Groups with Precise Control																
10–25 da. 0 to −4° C	62	26	25	11	12	5	12	4	3		49	6	5	1	1	19.0
10–25 days 20–31° C	49	1	5	43	0	5	22	15	6		5	13	17	10	4	13.0
Total March 11, 1931, to March 11, 1932																
10–25 da. 0 to −4° C	72	32	29	11	15	5	13	4	3		59	6	5	1	1	18.6
Total 20–31° C	87	1	14	72	0	10	40	26	10		12	20	30	19	6	12.7

The total group adds to those already given such animals as were submitted to similar conditions in other connections and had accordingly no precise controls. Those kept at 20 to 31° C include 23 kept in the light and 2 kept 5 days in the dark. Except when indicated, the stock diet with milk was used.

For key see Table I.

This process of hypertrophy and colloid loss was most extreme centrally, but in the more severe instances the poles were involved. When the larger peripheral follicles were affected the walls became irregular and plicated, a bulging capillary tunneling each epithelial crest (Figs. 1 and 5). In general, the vacuolation of the colloid was striking, and remnants in nearly empty follicles became granular in character. Mitotic figures were often present. In one series they were counted according to the technique of Gray and Loeb [16] of enumerating those seen in every tenth 10 micron section. Values of 35.4, 158, 223, 7.9, 39.1, 48 and 1.3 per mg. of gland were found, as compared to none in the 6 controls. The glands were commonly more vascular microscopically, although this is in many cases not easy to determine. In certain extremes the follicles are reduced to solid epithelial columns separated by dilated sinuses of blood (Fig. 5). Necrosis or desquamation of epithelium, described by Cramer, was notably absent save in two animals examined several hours after death, although collapsed injured follicles, not unlike those pictured by this author, may be seen at the periphery of any gland, whether the animal was exposed to cold or not, and due, presumably, to manipulation.

While the character of the changes was consistent throughout, the degree was not. In the experiments performed in the spring and summer of 1931 the hypertrophy and colloid loss frequently dominated the gland; in those performed in the late fall and winter of 1931–32 such changes, although consistent, were focal for the most part. This variation was especially striking with regard to weight. A 20 day experiment from June 12 to July 2, 1931, produced glands averaging 34.8 mg. per 100 gm. body weight for 7 animals, as compared to a control of 12.3; while a 21 day experiment performed between February 19 and March 11, 1932, produced glands averaging 14 mg. in 12 animals, as compared to a control of 12.4. As the controls for these two groups were microscopically similar, variation in the stock does not account for the differences. Such irregular results, unfortunately common enough in work on the thyroid, are not easy to explain but, as will appear later, may be due to uncontrolled variations in the iodine content of the diet or of the atmosphere.

The Effect of Iodine on the Changes Induced

Table IV gives the results on three comparative series, running from 15 to 21 days, comprising in all 50 animals, of which half received 10 y* of iodine daily in the form of potassium iodide dissolved in physiological saline and injected subcutaneously. The effect was to reduce materially the incidence of hypertrophy and colloid evacuation. Thus only 6 animals showed areas of marked colloid diminu-

Table IV

Showing the Influence of Iodine, 10 y Daily, on the Response of the Thyroid to Cold

Experiment	No. of animals	Amount of colloid									Height of epithelium					Average wt. of thyroid mg. 100 gm.
		0–1			0–1, 2	2	2, 3	3	3, 4		3, 4	3	2, 3	2	1, 2	
		Most	foci	absent												
July 5 to 20, 1931 — Stock Diet with Milk																
15 days at 0° C with iodine	6	1	1	4	0	1	3	1	0		1	3	2	0	0	8.5
15 days at 0° C no iodine	5	5	0	0	0	0	0	0	0		5	0	0	0	0	16.3
December 11 to 28, 1931 — Stock Diet with Milk																
17 days at −3° C with iodine	9	0	1	8	1	1	7	0	0		0	1	4	1	3	10.2
17 days at −3° C no iodine	8	1	5	2	1	3	3	0	0		4	2	1	1	0	16.3
February 19 to March 11, 1932 — Modified Diet with Water																
21 days at −4° C with iodine	10	0	3	7	0	1	7	1	1		0	5	5	0	0	11.7
21 days at −4° C no iodine	12	0	10	2	6	0	5	0	1		10	1	1	0	0	14.0
All Groups																
Cold + iodine	25	1	5	19	1	3	17	2	1		1	9	11	1	3	9.8
Cold only	25	6	15	4	7	3	8	0	1		19	3	2	1	0	15.2

For key see Table I.

* y = gamma 0.001 mg.

tion as compared to 21 receiving no iodine; 1 had high columnar epithelium as compared to 19 receiving no iodine; the average weight of the glands was 9.8 mg. per 100 gm. body weight as compared to 15.2.

The amount of iodine was much less than the daily 0.5 and 2 mg. KI observed by Rabinovitch [17] to increase mitotic proliferation in the rat when given for 5 to 10 days; indeed the mitotic proliferation induced by cold was suppressed. Thus, in one series 5 animals receiving no iodine had 8.2, 57.8, 10.9, 4.9 and 6.4 mitoses per mg. of gland, as compared to 0, 0, 0, 0, 0.9 and 14.4 for the 6 animals receiving iodine.

Two groups were compared, one kept in the cold and the other at room temperature, both receiving 10 y of iodine daily (not given in the tables). The effect of cold on the epithelium and colloid content was very nearly completely obscured, although the glands of the 7 animals kept in the cold averaged somewhat heavier, 13.6 as compared to 8.5 mg. per 100 gm. body weight.

The effect of iodine in partially or completely abolishing the transformation effected by cold may very well provide an explanation for the discrepancies between the work of Cramer and Mills on the one hand and those of Hart and Bergfeld on the other, by virtue of the inevitable variation in the iodine content of natural diets and the likelihood of iodine permeating the air of laboratories.

The Significance of Hypertrophy and Colloid Loss

The contentions of Cramer and Mills being herein largely borne out under circumstances in which the animal's metabolism is extraordinarily increased (approximately 150 per cent according to the careful studies of Benedict and MacLeod [18]), it is natural to conclude forthwith that the changes induced represent increased delivery of thyroid hormone to the blood stream. While the writer is inclined to this view, to state it so baldly is to ignore the subtle confusion which pervades the entire subject of the meaning of the hypertrophy of the thyroid cell. Some general considerations are hence necessary.

The structure of the anatomical unit of the thyroid as a closed cellular vesicle holding a thyroglobulin-containing colloid necessitates the view that this colloid represents the secretion of the thyroid gland over and above that being delivered to the tissues. This re-

mains true whether colloid formation is obligatory as a phase in the synthesis of the hormone, or whether, following Bensley,[19] it is an alternative to direct export of secretion from cell to circulation. For in either case, in an extremity, colloid is withdrawn.

Loss of colloid may therefore occur either as the result of an impairment of its formation or because of unusual excretion. Below is a schematic presentation of the various factors which may operate in either case. Although given abstractly, nearly every point has entered into someone's theory.

A. Impaired formation of colloid:
   1. Because the ingredients of the colloid are unavailable.
   2. Because the cells themselves suffer from some specific inability to form the colloid.
   3. Because some extrinsic mechanism stimulating colloid formation fails.
   4. Because some extrinsic mechanism inhibiting colloid formation overacts.

B. Increased export of the substance of the colloid or of a derived substance:
   1. Because the constitution of the colloid itself makes it impossible of retention (see A, 1 and 2).
   2. Because of increased stimulation by an extrinsic mechanism acting either (*a*) in accord with tissue needs, or (*b*) independent of them.
   3. Because of diminished inhibition by an extrinsic mechanism acting either (*a*) in accord with tissue needs, or (*b*) independent of them.
   4. Because of increased release of indigenous origin, as in neoplasms.

Now it is unfortunate that as yet no distinction can be made between A, impaired formation, and B, augmented excretion on cytological grounds. Hypertrophy and hyperplasia of the epithelium and increased vascularization accompany the process of colloid solution and loss in both instances. Exophthalmic goiter in man and the hyperthyroidism induced experimentally by anterior pituitary preparations at once suggest themselves as instances in which the excretion of the hormone is excessive; while in endemic goiter for all the intense hypertrophy and marked reduction of colloid there are no signs that the organism is being flooded with a recognizable thyroid hormone. The most modern iodine restriction studies furthermore present excellent evidence that when this essential element of the secretion is lacking, hypertrophy accompanies the colloid loss. Thus Levine [20] and Remington [21] in South Carolina have recently reported diffusely hyperplastic goiters, four times the normal size, occurring in white rats kept 35 days on a diet containing 15 parts of iodine per

billion, the stock diet having an iodine content of 42 to 72 parts per billion. This confirms the earlier work of McClendon and Williams,[22] Hayden, Wenner and Rucker,[23] Drennan, Malcolm and Cox,[24] and Krauss and Monroe,[25] and negates that of Hellwig[26] and Jackson and P'an,[27] who, unfortunately, have not provided actual analyses of the iodine-poor diets with which they worked.

A parallel to this discrepancy between hypertrophy and basal metabolism exists following administration of iodine to animals with diffusely hyperplastic goiters. There is always a recession of hypertrophy to some extent, however patchy, together with an accumulation of colloid, but the effect on the basal metabolism varies with the state of the animal. In human hyperthyroidism after the use of iodine there is a decline in the basal metabolism, often incomplete and transient but definite and important. Likewise, in the hyperthyroidism provoked in the guinea pig by those anterior pituitary preparations recently developed by Loeb and his coworkers,[28-30] Aron,[31] Schockaert,[32] Krogh, Lindberg and Okkels,[33] and others, iodine suppresses the greater part of the rise in the metabolism (Siebert and Thurston[43]). However, in the endemic goiter in the rabbit described by Chesney, Webster and Clawson[38, 44] in which the metabolism is primarily low, iodine induces a sharp, intense and often fatal hyperthyroidism,[45, 46] which takes place apparently while the cellular hypertrophy is receding and colloid is accumulating. This effect was confirmed by Marine (cited by Webster and Chesney[45]) who, curiously enough, had with Lenhart observed it years ago in goitrous dogs and made little of it.[48] It is presumably similar to the toxic reactions that halted the early iodine therapy of endemic goiter in man,[50] and which are now, with a few often doubtful exceptions, rarely seen in America in any precise and unmistakable form. Rienhoff[47] was quite unable to produce them by deliberately giving iodine to patients with nodular goiters. It may well be that these

NOTE: For many illustrations of hyperplastic thyroids occurring in endemic goiter, Marine[34] may be consulted; for a discussion of the disparity between the histological findings of intense activity of the thyroid and a normal general state of health in the dog, Carlson and Woelfel[35]; for the occurrence of the cretin, hairless pig in such stock, Hart and Steenbock[36] and Smith[37]; for a recent determination of low basal metabolism in the rabbit with diffusely hyperplastic endemic goiter, Webster, Clawson and Chesney[38]; for discussion of normal clinical states and normal basal metabolisms in human adolescents with diffusely hyperplastic goiters, Gold and Orator,[39] Wegelin,[7] de Quervain,[40] and de Quervain and Pedotti[41]; for the appearance of human cretinism in the presence of abundant hyperplastic thyroid tissue, Wydler.[42]

inconsistencies between present clinical experience and the Hopkins experiments are more apparent than real, and have as their explanation the present rarity in man, especially in America, of the massive diffusely or abundantly hyperplastic endemic goiter, which forms the anatomical substrate for the experimental iodine hyperthyroidism. No student has as yet been able to reconcile in a comprehensive theory these two apparently opposing phenomena — the stimulation of basal metabolism by iodine in certain endemic goiters, and the suppression of elevated metabolism in human exophthalmic goiter and in animal hyperthyroidism due to pituitary injection. It is sufficient for the present to indicate that both indubitably occur and that as with hypertrophy and evacuation of colloid, recession of cellular enlargement and accumulation of colloid cannot measure precisely the amount of thyroxin being delivered to the organism.

To Marine,[34, 48, 49, 50] speaking from his experience with the resolution of simple goiter by iodine, we are indebted for the forcible presentation of the idea that hypertrophy expresses a relationship between supply of hormone ingredients (stated in terms of iodine) and excretion of hormone, rather than simply augmented excretion of hormone from the gland. Unfortunately, however, this author's use of two terms, "work hypertrophy" and "compensatory hypertrophy," in connection with the reaction to iodine insufficiency, has contributed confusion: the former term because, while conveying properly enough the impression of physiological strain that enlarging and multiplying cells must always give, implies a physiologically significant hypersecretion, of which there is no evidence; while "compensatory hypertrophy," when the gland substance has not been actually reduced, substitutes a vague teleology for specific anatomical meaning which, in the end, must be found for so regular, common and clear-cut a phenomenon. Plummer [51] has, with characteristic subtlety, suggested that the hypertrophied thyroid cell may always be excreting, under stimulation, an excessive amount of fluid menstruum which varies, however, in its thyroxin content from the large amount present in exophthalmic goiter to virtual absence in certain cretins.

Tentatively, then, for the purpose of the interpretation of the work herein presented, a view not far from those of Marine and Plummer will be adopted to the effect that hypertrophy with declining colloid content represents preponderating excretion of the hormone from

the gland, whether the amount excreted be great or small. Such hypertrophy may result from either insufficient material to form the hormone needed or from excessive export of the substance formed. The distinction between these two can only be made by: (*a*) demonstrating the absence or unavailability of some known ingredient of the secretion, or (*b*) recognizing the effects of thyroxin operating in the organism to a greater or less degree, or in some future time measuring the thyroxin content of the blood.

The Interpretation of the Effects of Cold

The significance of the hypertrophy and colloid loss regularly observed in the thyroid gland when rats are exposed to cold for 10 days or more can only be established when it is known that the essential ingredients of the colloid are being adequately supplied and that there is an increased demand for the hormone under these conditions.

There are no good grounds for supposing that the animals are deprived of any essential ingredient of the hormone. They eat more dry food than their controls, the process occurs when the milk intake is not seriously limited by freezing (7 animals, 25 days — August 24 to September 18, 1931; 5 animals — April 15 to 26, 1931), or when dried milk is used and the fluids supplied as water (Diet 2, 12 animals 21 days — February 19 to March 11, 1932); so that it is unlikely that any known food constituent is absolutely restricted in the experimental animals as compared to the controls, although it is theoretically possible that some essential substance might be used in large amounts by the active muscles and hence deviated from the gland. As for iodine, any loss due to freezing of water (using McClendon's estimate of 0.67 part in one billion of 68th Street crib Lake Michigan water) is made up many times by the ingestion of additional food that must contain much more than the 15 parts per billion found goitrogenic for the rat by Levine and Remington or the 10 parts per billion in McClendon's most iodine-poor regimen.

That the capacity of the cells to form the hormone was not impaired may be judged from the normal cytology, and from the fact that iodine so completely prevented the change.

The heightened tissue activity induced by exposure to cold includes increased muscle work plus any such direct stimulation of basal metabolic processes as may take place, these two phenomena

occurring in completely unknown proportions at this intensity of cold. Benedict and McLeod, after carefully studying activity records of white rats at 21 and 28° C, concluded that differences in heat production could not be accounted for by the differences in the recordable movements of the animals. Furthermore, rats kept at 21° for 24 hours have a slightly higher basal metabolism, measured at 28°, than when kept for 24 hours at 28° (4.5 per cent in all but 1 of 11 animals). The quantitative importance of this suggested increase in basal metabolism induced by cold at such temperatures as alter thyroid structure and the actual rôle of the thyroid in its production remain undetermined.

It is natural enough to relate thyroxin utilization to the augmented total metabolism induced by cold. Cramer, Ludford and Mills have done this and are in accord with one of the most popular conceptions of the action of the thyroid hormone, that of Plummer, to wit: "Thyroxin is a catalyst hastening the rate of formation of a quantum of potential energy available for transformation on excitation of the cell." The great difficulty here rests in the uncertainty concerning the place of thyroxin in the metabolism of active muscle. Boothby, Buckley and Wilhelmj [52] have recently, in the dog, found the calorigenic action of thyroxin greater during a period of work in a treadmill than before the work was started. If thyroxin were used up by the active muscles its calorigenic action should have been less during work. While, as recognized by these workers, this experiment would have been more conclusive if performed on thyroidectomized animals, it does create at present a substantial obstacle to the relating of the function of thyroxin in a simple quantitative way to muscular oxidative activity in general. On the other hand, several recent physiological studies [53-56] have concurred in the finding that the neuromuscular system of the heart removed from central nervous control responds to thyroxin by increased contraction. Of course a system with natural rhythmicity is metabolically different from one that contracts only with extrinsic stimulation, so that we should not make too much of these discrepancies. Suffice it to say, however, that we may not as yet conclude directly from increased total metabolism that more thyroxin is required. It is necessary to demonstrate that the increase in heat production in the thyroidectomized animal on exposure to cold is less than that of the normal. Two experiments, neither conclusive, are available on this point.

Hildebrandt in 1921,[57] using the white rat and a modification of the Haldane technique, compared the basal metabolism for 2 hours in an air chilled chamber at 4 to 6° C with that in a warm room at 19 to 20° C. In 3 animals there was no difference before and 4 to 8 days after thyroidectomy. One animal studied 32 to 45 days after thyroidectomy showed only a slight rise at the low temperature, but not less than some normals. Isenschmid in 1923,[58] using the Haldane technique, found in 2 rabbits that 5 days after thyroidectomy there was no less response during 2 hours at a temperature of 8 to 9° C than before. In both of these reports the number of animals used is too small, the time after thyroidectomy usually too brief for the animal to be rid of its thyroxin, and the duration of the experiments insufficient to permit the realization of the effects of a substance with so long a latent period as the thyroid hormone.

Thus the physiological evidence for increased thyroid secretion during exposure to cold remains far from satisfactory.* Until better data on this point are forthcoming the interpretation of these experiments must be qualified. The cellular hypertrophy and loss of colloid regularly observed in the thyroid glands of rats exposed to cold represent a preponderant excretion of hormone from the gland; there is no evidence that formation of hormone is impaired, but we must await the demonstration of the greater need for thyroxin by the active tissues before accepting as a strict scientific inference the conception that under these circumstances there is an augmented excretion of the hormone from the gland.

The modifying influence of iodine may similarly be looked upon as meaning an increase of hormone formation relative to excretion and not as necessarily indicating a reduction of the output of the hormone from the gland.

Summary and Conclusions

1. Exclusion of light for periods of 10 to 25 days did not produce any striking changes in the thyroid gland of the rat.

2. In confirmation of the work of Cramer, Ludford and Mills, exposure of rats to cold for 10 to 25 days produced hypertrophy,

* Interesting correlative evidence is presented by Dietrich and Schwiegk[59] who demonstrated recently in the anesthetized dog that chilling the animal increases the blood flow to the thyroid.

hyperplasia, loss of colloid and increased vascularity in the thyroid gland.

3. Iodine in doses of 10 γ daily diminished or prevented this change.

4. Hypertrophy and loss of colloid in general mean preponderant excretion of the hormone from the thyroid gland, either arising from impaired formation or from excessive excretion of the hormone. The actual amount excreted must be determined on physiological grounds.

5. There are no good reasons for supposing that impaired formation of hormone occurred in these experiments.

6. It is likely that the hypertrophy and colloid loss herein described signify that the thyroid gland was excreting more of its hormone, but the essential demonstration of increased need by the tissues for thyroxin is as yet lacking.

The writer wishes to express appreciation for the stimulation and considerate counsel of Drs. A. J. Carlson, Louis Leiter, O. H. Robertson, Irene Sandiford, Paul Starr and Russell M. Wilder at various points along the way.

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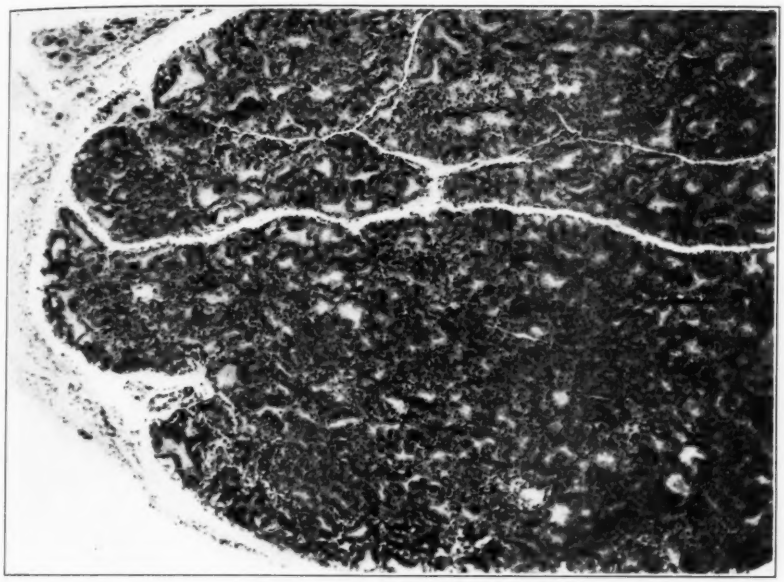
DESCRIPTION OF PLATES

PLATE 59

FIG. 1. Rat 169, kept 25 days at −1° C. Thyroid gland showing hypertrophy of cells and loss of colloid. × 70.

FIG. 2. Rat 177, litter-mate of 169, kept 25 days at 28° C. Normal gland. × 70.



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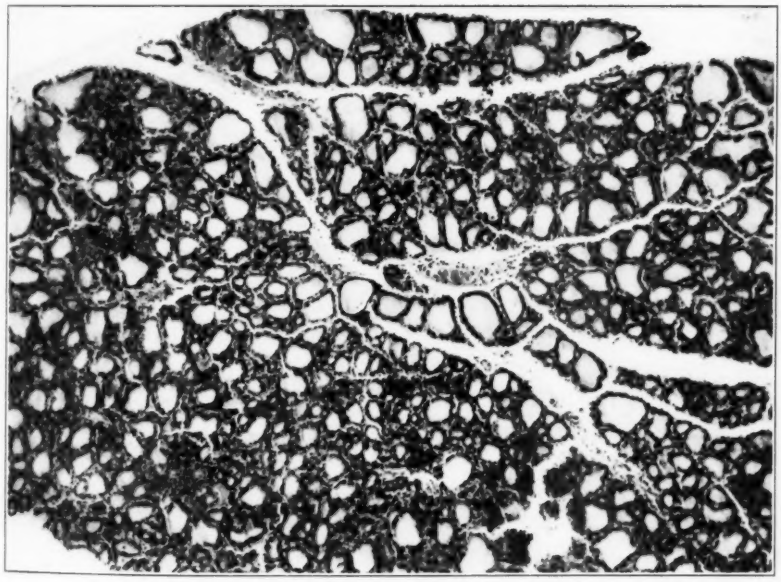


2

Kenyon

Changes in Thyroid Gland of Rat Exposed to Cold

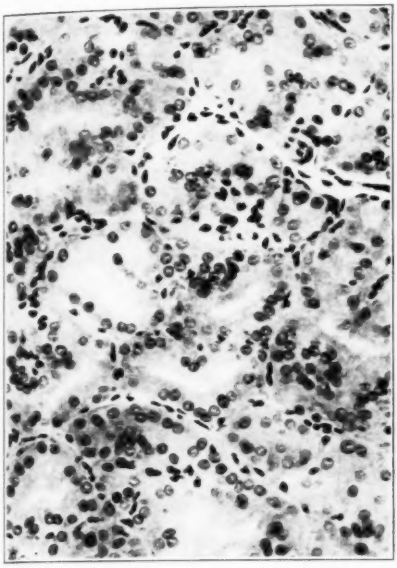
PLATE 60

FIG. 3. Rat 160, kept 25 days at −1° C. Thyroid gland showing hypertrophy of cells and loss of colloid. × 275.

FIG. 4. Rat 177, litter-mate of 160, kept 25 days at 28° C. Normal gland. × 275.

FIG. 5. Rat 172, kept 25 days at −1° C. Thyroid gland showing the folding of large follicles with the disappearance of colloid, together with the distention of the vascular channels. × 275.

FIG. 6. Rat 317, kept 21 days at −4° C, but receiving iodine 10 γ daily as potassium iodide. The marked hypertrophy and loss of colloid does not occur. × 275.



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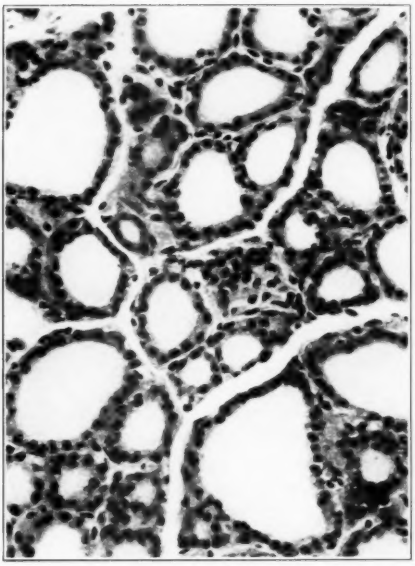

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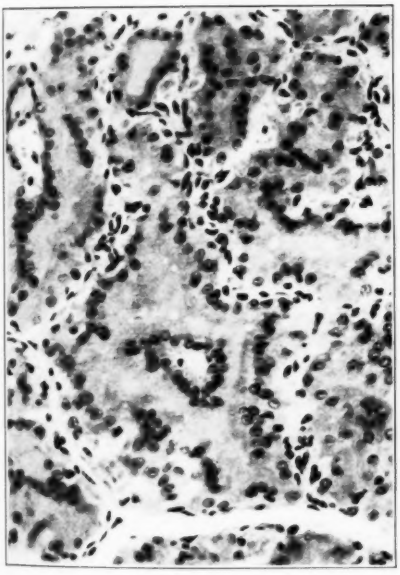

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6

Kenyon Changes in Thyroid Gland of Rat Exposed to Cold

FIBROSIS UTERI *

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This paper presents a study of the collagenous tissue of the myometrium at different age periods and in association with various pathological conditions. An attempt has been made to distinguish the effects of disease from alterations that are merely the result of age. The relation of uterine hemorrhage to fibrosis of the myometrium is discussed.

The relation of fibrosis of the myometrium to uterine bleeding has long been a moot question. Numerous definitions of "fibrosis uteri" have been offered. Shaw (1914) gave a very broad definition: "A uterus enlarged, hard, and containing no tumors, which causes chiefly hemorrhage, pain, leukorrhea, or a combination of these." Bland-Sutton (1904) gave the following description: "A peculiar affection of the uterus, of which the leading clinical feature is uncontrollable menorrhagia, against which both drugs and curettage are of no avail. The disease is chiefly met in women between 35 and 45 years of age. The uterus is large, thick, and tough. Microscopically the muscle tissue is replaced by an abnormal growth of fibrous tissue. The walls of the uterine arteries are thick and the lumina of the vessels narrowed and occasionally obliterated."

The first article on this subject was published by von Scanzoni (1861), who described fibrosis as an anemic, dry, yellow, hard uterus with the arteries and veins reduced in caliber. Finn (1868) and later, Birch-Hirschfeld and Klebs (1896) claimed that the increase in the bulk of the fibrotic uterus was due to muscle tissue, while Klob (1868) insisted it was a direct result of hyperplasia of fibrous tissue. Olshausen (1875) first ascribed uterine hemorrhage to chronic infection of the uterine mucosa, and Brennecke (1882) pointed out the probable relation of the ovary to menorrhagia.

Fritsch (1885) attributed idiopathic uterine bleeding to the pathological increase of fibrous tissue in the uterine wall, which he regarded as the direct result of prolonged inflammatory reaction. Reincke (1897) and Cholmogoroff (1900) stated that uterine bleed-

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ing is due to changes in the blood vessels that have lost their contractile and retractile powers, while the uterine wall changes (fibrosis) are coincidental or secondary. Findley (1905), the first American to study this subject, agreed with the above authors.

Solowij (1907), however, insisted that the myometrial changes are first and the arterial changes secondary. Theilhaber and Meier (1902) and Rabinovitz (1910) agree with Solowij and conclude that uterine bleeding is due to a condition of "insufficienta uteri." This is caused by an intermuscular and perivascular development of connective tissue and a corresponding decrease of the muscular elements, decreasing the power of the uterus to contract and control bleeding. Arterial changes are secondary and endometrial changes are unimportant. Theilhaber suggested the possible influence of the ovaries on this condition.

Gardner and Goodall (1906) divided chronic metritis into two types: (*a*) the simple type in which only the uterus is concerned, and (*b*) the complicated type, with other organs involved. The bleeding in simple metritis was attributed to two conditions. Corroborating Theilhaber, they believe it is due to a muscular insufficiency of the uterine wall and the arteries. The insufficiency is due chiefly to the restraining influence of the increased amount of non-contractile tissue. This increase of fibrous tissue may be the result of subinvolution, infection, any prolonged congestion or chronic irritation of the uterus such as pregnancy, gestation, adnexal disease, and so on. The second cause of simple metritis is arteriosclerosis.

Rabinovitz (1910) believed that the arterial changes in fibrosis uteri are not arteriosclerosis since the change usually does not begin in the intima. The growth of new connective tissue is seen around the adventitia into which it finally extends. The media undergoes a muscular hypertrophy, probably compensatory, while the intima shows no deviation from the normal. The lumen is held open, while in arteriosclerosis it is closed. Solowij agrees with Rabinovitz. Healy (1915) described two types of arteriosclerosis in women: the one with general arteriosclerosis, involving the heart, liver, kidneys, and so on, leading to a congestion of the pelvic organs from obstruction of the circulation and causing bleeding; the other, with arteriosclerosis of the uterine vessels only. Healy, however, does not discuss the structural alterations in the uterine wall with the above conditions.

Pankow (1909) was the first to doubt the causal relationship between menorrhagia and fibrosis of the myometrium. He proved that myometrial changes may occur without bleeding. Chalfant (1911) after studying eleven cases of "idiopathic" bleeding agrees that microscopically one cannot tell a bleeding from a non-bleeding uterus. Donald (1914) concluded that, aside from the great thickness of nulliparous uteri, there was no difference between the normal and the so-called "fibrosis uteri."

Whitehouse (1914) described a case of fibrosis uteri which he attributed to syphilitic infection. A single instance, however, does not establish a causal relationship between these conditions.

Shaw (1914) believed that the tremendous increase in elastic tissue usually found in multiparous uteri was the most important cause of menorrhagia. He thought that the sheets of elastic tissue about the vessels interfered with their contractility and therefore favored bleeding. He ascribed the bleeding in the nulliparous uterus to endometrial changes.

Geist (1915) after examining 25 specimens with the diagnosis of "fibrosis uteri" decided that he could not find any definite histological lesion to account for the profuse bleeding.

Material and Technique

For this study 40 uteri obtained at autopsy and 19 uteri removed at operation were examined. This material was divided into the following groups.

The 13 uteri in the first group were from nulliparas ranging in age from 17 to 88 years, dead of accidental causes. In these cases there was no history of local or systemic disease and no lesions were found postmortem, except those due to the fatal accident. There was also no history of abnormal uterine bleeding.

In the second group there are 7 uteri from nulliparas dead of acute infectious processes, *i.e.* pneumonia, diphtheria, liver abscesses, septicemia, and so on. The ages ranged from 23 to 65 years. There was no history of abnormal uterine bleeding in these cases.

In the third group of 14 cases death was caused by a chronic disease, *i.e.* tuberculosis, tertiary syphilis, chronic alcoholism, and so on. The ages in this group ranged from 19 to 78 years. All were nulliparas without a history of abnormal uterine bleeding.

The 10 uteri of the fourth group were operative specimens removed from nulliparas 19 to 45 years of age because of acute pelvic inflammation.

The fifth group includes 15 uteri (9 operative and 6 postmortem specimens) in which there was a history of abnormal bleeding. Six were from nulliparas 21 to 60 years of age, while 9 were from multiparas whose ages ranged from 26 to 53 years and who had had from 2 to 13 pregnancies.

Blocks were taken from various parts of the uterine wall and fixed in both formalin and Zenker's solutions. All sections were stained routinely with Weigert-Van Gieson, azocarmine, Weigert's elastic tissue, and hematoxylin-eosin. For the study of the fibrous tissue in the myometrium and its arteries the azocarmine stain is invaluable, since it demonstrates even the smallest connective tissue fibers. However, it does not differentiate between elastic and collagenous fibers, which is very important in the study of the parous uterus. For this, Weigert's elastic tissue stain or, better still, the combination of Weigert-Van Gieson serves very well. Hematoxylin-eosin, Van Gieson, and the Dominici stains were used to study the cellular structure.

The Myometrium of a Normal Nulliparous Uterus of a Young Woman

As early as 1889 Dittrich criticized the work of the earlier writers because in their studies they did not exclude pathological processes. To avoid this objection the criteria of normal structure have been established by the study of 7 uteri of young women dead of accidental causes, whose histories revealed no recent acute infections, no chronic infections, and no pelvic diseases. The ages varied from 17 to 26 years.

It is with the myometrium that we are principally concerned. In lower mammals this layer can be divided into three distinct strata; however, in the human species the arrangement of muscle fibers is much less regular and the wall cannot be separated into definite subdivisions, although careful examination reveals three indistinct layers intimately blended with one another. The outermost of these is relatively thin, consisting mostly of longitudinal, but also of some circular and oblique bundles. The second is a thick layer of inter-

lacing bundles of more or less circular fibers. Intermingled with these are many large blood vessels supplying both the mucous and muscular coats and giving to this layer the name "stratum vasculare." It merges into the third and innermost layer, stratum submucosum, which also contains many longitudinal fibers.

In the myometrium the smooth muscle fibers are arranged in flattened or cylindrical bundles, separated from each other by bands of connective tissue that extend between the individual muscle cells (Fig. 1). In cross-section the muscle fibers are round, their nuclei are large and well defined, and contain many nucleoli. In longitudinal section it is very difficult to make out the individual muscle fibers with routine stains; but the nuclei appear extremely elongated in the long axis of the fibers, have rounded ends, and contain several nucleoli. There is much variation in the amount of connective tissue between the muscle bundles and muscle fibers. Since in both the outer and inner portions of the myometrium there is a slightly increased amount of connective tissue, the differentiation of the individual muscle fibers is much easier (Fig. 1). In the middle layer, however, the separating tissue is greatly diminished and the muscle cells, which seem to fuse with one another, are practically indistinguishable with the ordinary stains and often even with the Weigert-Van Gieson. However, with the azocarmine the individual muscle fibers, even in the thick middle layer of the myometrium, are well defined by the thin strands of deep blue-staining connective tissue between them.

In the somewhat looser connective tissue between the thicker bundles of smooth muscle, especially in the inner and middle strata of the myometrium, various cells are found. With the Van Gieson and hematoxylin-eosin stain some appear as long flat bodies with several broad, smooth-edged processes, and large oval nuclei with fine chromatin particles and deeply stained nucleoli. These are undoubtedly fibroblasts. There are also some smaller cells, with large dark nuclei and a thin zone of cytoplasm, that resemble lymphocytes. Still other cells of irregular shape are seen that have small kidney-shaped nuclei with a dark coarse chromatin pattern and without nucleoli. These may be called histiocytes. It has been shown by Maximow, Bloom, and Stieve that these three forms of connective tissue cells are not fixed types, but are to some extent interchangeable. During pregnancy there is an increase in the number

of connective tissue cells and then only can the different transition stages from the small (lymphocyte) to the large mononuclear (histiocyte and fibroblast) be seen. Stieve maintains that during gravidity connective tissue cells may become transformed into muscle fibers.

Between the individual smooth muscle cells there is seen in addition to the thin collagenous fibers a dense network of elastic fibers that branch irregularly and pass between the bodies of the muscle cells, forming a fine sheath about each. The amount of elastic tissue in the myometrium diminishes from the periphery toward the mucosa.

Age Changes in the Nulliparous Uterus

It is difficult to obtain uteri in which the alterations are due entirely to age, but 13 cases were available in which death was caused by accident. In these cases the ages varied from 17 to 88 years.

With the advance of age there occurs within the uterine wall a gradual increase in fibrous connective tissue that replaces much of the muscle. The uterine wall of a middle-aged nullipara is quite distinct from that of a younger woman (Figs. 2 and 3). Each individual muscle fiber, even in the muscular middle layer of the myometrium, is definitely enveloped by a heavy sheath of fibrous tissue. The collagenous fibers surrounding the muscle bundles have also increased greatly. The cells, so prominent in the loose connective tissue of a young woman, have disappeared to a great extent and have been replaced by collagenous fibers. The elastic tissue, however, seems unchanged in quantity and distribution. The arteries of the uterus of a middle-aged woman show very little change. The only consistent alteration in the arteries is an increase in the thickness of the adventitia. The muscle fibers of the media are not replaced by collagenous fibers; neither is there any change in the internal elastic lamina. Another minor variation, seen occasionally, is an increase in thickness of the musculo-elastic layer between the elastica interna and the endothelial lining.

In middle life the uterine wall is already beginning to assume the appearance (Fig. 3) so frequently described by many authors as "fibrosis uteri," a change that is found in the myometrium of every older nullipara, regardless of whether disease has been present or not.

The myometrium of an aged nullipara shows still more marked differences (Fig. 4). At this stage the fibrous tissue has increased to such an extent that almost all the muscle fibers have been replaced. It is difficult to differentiate between interfascicular and interfibrillar connective tissue, since they have more or less fused, obliterating the outlines of muscle bundles. The entire uterine wall appears blue with the azocarmine stain. The muscle nuclei are visible after the sarcoplasm has practically disappeared.

The arteries show much less pronounced age changes than the myometrium. The adventitia of the arteries is greatly thickened and blends with the surrounding fibrous tissue. The media and adventitia are no longer separable, because of the replacement of the media by collagenous fibers. Many arteries are seen, however, in which there is no medial fibrosis. With the azocarmine stain these arteries stand out conspicuously as pinkish structures in a solid blue background. Occasionally in the old nullipara there is found medial calcification of the uterine arteries. The intima remains surprisingly free of change in many of the arteries, although the majority show moderate intimal atherosclerosis.

What effect do disease processes have on the age changes in the nulliparous uterus? Seven uteri were examined from nulliparas dead of acute infections: 3 aged 23, 41 and 50 years, dead of pneumonia; 1 aged 30 years, dead of diphtheria; 1 aged 54 years, dead of multiple abscesses of the liver; and 2 aged 42 and 65 years, dead of septicemia. In none of these specimens were there any tissue changes that would enable one to differentiate them from the normal nulliparous uterus. Ten uteri that had been removed because of acute pelvic inflammatory disease were examined and these also were found to be undifferentiable from the normal. It appears, therefore, that acute infectious diseases have absolutely no effect on the uterine tissue, be it a generalized process such as diphtheria or pneumonia, or a localized condition such as an acute pelvic inflammation.

The chronic diseases, however, such as tuberculosis, syphilis and alcoholism do tend to alter the normal physiological age changes. Fourteen uteri of nulliparas dead of various chronic diseases were studied: 3 aged 31, 49 and 78 years, dead of long standing cardiac disease; 5 aged 19, 21, 23, 49 and 50 years, dead of chronic tuberculosis; 4 aged 21, 25, 31 and 55 years, dead of tertiary syphilis; and 2 aged 27 and 50 years, dead of chronic alcoholism. The myometria in

the young nulliparas who had suffered from long-standing chronic diseases did not differ microscopically from those of young healthy virgins. The uterine walls of the older nullipara, however, were a great deal more fibrous than those of the healthy nulliparas of the same age. It would appear that, although chronic diseases have no apparent effect on the uterine wall of a young nullipara, they hasten the normal age fibrosis in the older nullipara.

The Relation of Fibrosis Uteri to Uterine Bleeding

A discussion of the collagenous tissue changes in the myometrium leads naturally to a consideration of the relation of fibrosis uteri to uterine bleeding. For many years hysterectomies have been performed for uterine bleeding, and in the absence of other changes the bleeding has been attributed to fibrosis of the myometrium. However, several investigators have suggested that fibrosis uteri is chiefly an aging process in the uterine wall and that there is no satisfactory evidence that it is responsible for uterine hemorrhage.

In order to study this problem 15 uteri, in which there was a history of unexplained uterine bleeding, were carefully examined grossly and microscopically. The azocarmine stain proved invaluable in this study because it brings out sharply all the connective tissue fibers in the myometrium.

A study of the uteri from cases of idiopathic bleeding in young women showed no increase of fibrous tissue or abnormal arterial changes that would distinguish them from the normal controls. In the elderly women there was often moderate to extreme fibrosis and a varying degree of arterial change, but these alterations were not more prominent than in the non-bleeders of corresponding age (Figs. 3 and 4). My observations, therefore, support the view that fibrosis uteri is not responsible for uterine bleeding.

Shaw suggested that the increase of elastic tissue around the uterine vessels of the multiparous myometrium interfered with their contractility and was the most important cause of menorrhagia. From the foregoing discussion it is apparent that the predominant change in the nulliparous uterus is an increase of collagenous tissue, but in the parous uterus the outstanding alteration is in the elastic tissue. In microscopic sections of a typical multiparous uterus one readily notes a striking increase of elastic tissue throughout the uterine musculature generally, but more particularly about the

arteries. This tissue is derived primarily from the old uterine arteries during the process of involution, and its amount varies with the number of pregnancies, the health of the individual during her gravid period, and the time of sexual life during which the pregnancies occurred. Szasz-Schwartz (1903), Pankow (1909), and Goodall (1910) have described these elastic tissue changes in the multiparous uterus.

It is evident from microscopic observation that all multiparous uteri have an increase of elastic tissue, but all women who have had children are not afflicted with menorrhagia. In this study it is seen that the myometria of multiparas removed because of uterine bleeding did not contain an excess of elastic tissue over the normal multiparous controls.

The size of the uterus bears some relation to its microscopic structure. The uteri of young nulliparas are of normal size, but a gradual enlargement occurs in middle life as fibrosis of the myometrium develops. However, in the senile period when fibrosis is most marked the uterus decreases in size, finally becoming small and brittle — the typical senile uterus. It is a common belief that the typical example of "fibrosis uteri" is an enlarged organ. This opinion is probably due to the fact that most of the uteri removed because of bleeding are in the physiological stage of enlargement.

Summary and Conclusions

1. With the advance of age, there occurs within the uterine wall a gradual increase of collagenous fibers that ultimately replace the greater part of the muscle.

2. Neither localized nor systemic acute processes have any effect on the uterine wall.

3. Chronic diseases have no effect on the uterine wall of young nulliparas, but hasten the age fibrosis in older nulliparas.

4. The uterine arteries show less pronounced age changes than the uterine musculature. The three alterations most commonly found are (1) medial fibrosis, (2) medial calcification, and (3) intimal atherosclerosis.

5. There is no satisfactory evidence that either fibrosis of the uterine wall or increase in elastic tissue, following pregnancy, is responsible for uterine hemorrhage.

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DESCRIPTION OF PLATE

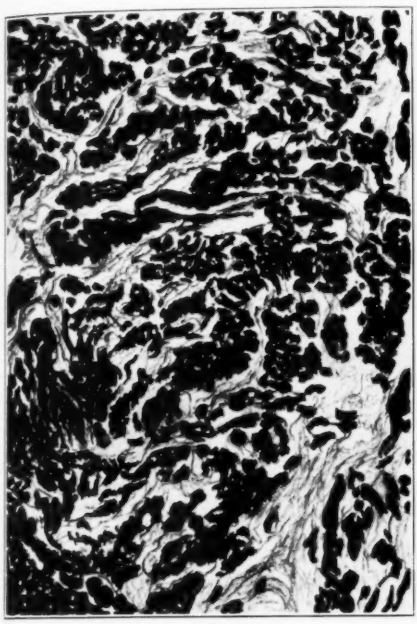
PLATE 61

FIG. 1. Drawing showing a portion of the outer looser layer of the myometrium in a normal healthy nullipara aged 17 years. Azocarmine stain. Note the predominance of the muscle tissue. Fine connective tissue fibers are seen between the muscle bundles. No history of abnormal uterine bleeding. In all four drawings muscle fibers are shown in black, and collagenous fibers in gray. × 500.

FIG. 2. Drawing showing a portion of the dense middle layer of the myometrium in a normal healthy nullipara aged 30 years. Azocarmine stain. Note the beginning increase of both interfascicular and interfibrillar connective tissue. No history of abnormal uterine bleeding. × 500.

FIG. 3. Drawing showing a portion of the dense middle layer of the myometrium in a normal healthy nullipara aged 42 years. Note the marked increase of collagenous tissue and its replacement of the muscle. No history of abnormal uterine bleeding. × 500.

FIG. 4. Drawing showing a portion of the dense middle layer of the myometrium in a normal healthy nullipara aged 72 years. Note the complete fibrosis of the uterine wall. There was no history of uterine bleeding. × 500.



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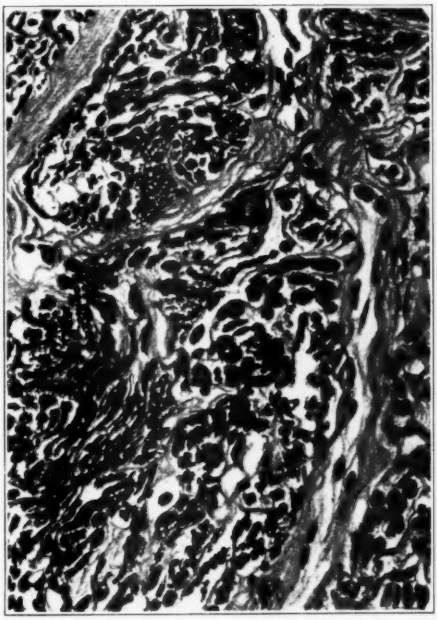

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Baker Fibrosis Uteri

HEMOPHILUS HEMOLYTICUS ENDOCARDITIS *

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Since the publication in 1923 by Miller and Branch [1] of a case of bacterial endocarditis caused by the hemolytic hemophilic bacillus, a bacterium that is normally non-pathogenic, no new cases of endocarditis caused by this organism have appeared in the literature. Russell and Fildes [2] reported a case of endocarditis caused by the so-called *Bacillus parainfluenzae*, an organism that more closely resembles *Hemophilus hemolyticus* than the ordinary strains of the Pfeifer bacillus. Neither the latter nor the *Bacillus parainfluenzae*, however, is hemolytic.

The hemolytic hemophilic bacillus was first described by Pritchett and Stillman [3] in 1919 in an investigation of the bacteriology of the then pandemic influenza, and was labelled by them "Bacillus X." This microörganism is morphologically and culturally similar to the Pfeifer bacillus, but differs in its ability to hemolyze red blood cells. Stillman and Bourn [4] recovered the bacillus from the sputum of patients suffering from acute influenza and lobar pneumonia and from the throat and saliva of healthy persons.

We quote from Miller and Branch a summary of the characteristics distinguishing this microörganism from the influenza bacillus: (*a*) it causes hemolysis; (*b*) it occurs only as a saprophyte; (*c*) it is less strictly dependent on hemoglobin; (*d*) it has a coarser morphology; and (*e*) it is slightly more difficult to keep in culture. Bergey [5] suggested the name *Hemophilus hemolyticus*, which we shall employ in the present discussion.

We shall proceed to the report of a case of bacterial endocarditis complicated by pregnancy, in which the blood culture yielded *Hemophilus hemolyticus* and also *Streptococcus viridans*. The presence of the former organism was also demonstrated in the microscopic lesions postmortem.

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Report of Case

Clinical history: The patient, F. F., a married female 32 years of age, an office worker, born in Czechoslovakia, was admitted to the Fourth Medical Division of Bellevue Hospital on November 22, 1931 with a complaint of chills, fever and cough of 3 weeks duration. The patient stated that she had enjoyed good health until the onset of the present illness, which was sudden and occurred about the evening of November 1, when she was taken with a severe chill lasting about 4 hours, followed by fever and sweating. She was prostrated the next morning and called her family physician, who treated her at home for 3 weeks. Her symptoms during that time were chills, fever and cough. The patient had had dyspnea on exertion and occasional edema of the ankles for the past 4 years. There was no previous history of rheumatic fever, chorea, scarlet fever or acute tonsillitis. Her last menstrual period she believed was about 3 months before admission.

Physical Examination: The patient was acutely ill, flushed, dyspneic and slightly orthopneic. Petechiae were present in the right conjunctiva and in the mouth. The teeth were carious and the gums showed marked gingivitis. The heart was not enlarged, the rate was rapid (120) and the rhythm regular. There was a soft blowing systolic murmur at the apex that was transmitted to the axilla. This was heard upon admission and thruoghout the course of illness. The chest findings consisted of dullness and bronchial breathing over the right lower lobe and a friction rub over the left lower lobe. The spleen was not palpable. The fingers showed slight clubbing and cyanosis. There was slight pretibial edema. On vaginal examination the uterus was felt to be enlarged to the size of a 4 months' pregnancy.

Laboratory Findings: Temperature 103° F, pulse 120, blood pressure on admission 90/60. The urine contained 2 plus albumin and a few red cells per high power field. Hemoglobin 50 per cent, red cells 3,500,000, color index 0.75, leukocytes 12,000, polymorphnouclears 75 per cent, and lymphocytes 25 per cent. Wasserman negative, blood chemistry essentially normal.

Course of Illness: The temperature was of the septic type — 103–4° F in the evenings with morning remissions to about 100° F. Pulse was always rapid, out of proportion to temperature. Blood pressure determinations remained fairly constant, about 120/80. Repeated urine analyses showed 2 plus albumin and casts. On one occasion red blood cells were noticed in the urine.

On November 24 the spleen was enlarged to percussion and on December 1 it became palpable for the first time. New crops of petechiae were discovered almost daily. A friction rub persisted over the left lower lobe. Numerous moist râles were heard from time to time, particularly over the right chest.

On the evening of December 3 the patient had a severe chill and the temperature rose to 105.4° F. New petechiae were noticed in the left conjunctiva. The patient was unable to move her legs and femoral pulsations were absent. A diagnosis was made of a saddle thrombus at the bifurcation of the aorta. Next morning both legs were cyanosed but warm, the body temperature dropped to 95° F and the patient appeared in a terminal toxic state. She died rather suddenly shortly thereafter, the temperature having meanwhile risen to 98.4° F.

The final clinical diagnosis was subacute bacterial endocarditis, saddle thrombus at bifurcation of aorta, and pregnancy.

Bacteriology

Three blood cultures were taken during life. The first taken on November 24 was negative after 5 days. On November 30 blood was withdrawn and cultured aerobically in dextrose broth, anaerobically in vitamin broth and in plain agar plates. The plates remained sterile for 10 days. After 48 hours spreads from the dextrose broth were stained and examined daily for 10 days. No organisms were obtained in any of the spreads. The anaerobic vitamin broth was examined on the 7th day and smears revealed an occasional small Gram-positive diplococcus. Subcultures were then made on blood-streaked agar plates. Growth appeared on the 4th day as thin, green, non-hemolytic colonies which, when transplanted to vitamin broth, grew in long chains. Blood plates streaked from these broth cultures showed typical colonies of *Streptococcus viridans*.

On December 3 blood was cultured as follows: 5 cc. were placed in each of two vitamin broth flasks and in one dextrose broth flask, and 1 cc. in each of three plain agar plates. The plates yielded no growth after 10 days. Smears of each broth culture were made after 24 hours and showed small Gram-negative coccoid bacilli occurring singly and in pairs. Some of the bacilli stained irregularly, showing slight polar bodies. Cultures in broth at 48–72 hours showed larger, non-mature Gram-negative bacilli.

Subcultures of the broth cultures were made on blood-streaked agar plates, blood-drop dextrose agar, ascitic agar and plain agar plates. No growth was obtained in the two latter. At 48 hours the two former showed several pin-point colonies, each surrounded by a small hemolytic area. After 72 hours the zone of hemolysis became much larger. On blood-drop agar plates the colonies were "influenza-like," minute, round, smooth, transparent and finely granular with a coarse, granular, central heaping. Growth on "chocolate slants" was abundant, gray and moist. No growth of transplants was obtained on plain or ascitic agar slants. Sodium oleate agar containing 1 per cent heated blood showed large, spreading, brown, moist, influenza-like colonies. When they were smeared the organisms appeared larger, were Gram-negative, and occurred in short filaments, pairs and groups.

POSTMORTEM EXAMINATION

Autopsy was performed within 4 hours of the patient's death. The anatomical diagnoses were: pericardial effusion, vegetative endocarditis of mitral valve, focal embolic myocarditis of left ventricle; infarcts of kidney and spleen; thrombosis of both iliac arteries; pregnancy estimated to be about 6 months; a Meckel's diverticulum; petechial hemorrhages in skin, conjunctivae and pericardium.

Autopsy: The body is that of a white female about 35 years of age with a well developed frame in a poor state of nutrition. Reddish purple petechiae are present over the right shoulder, neck and in both conjunctivae and sclerae. The left leg and thigh are deep purple in color. Both legs are slightly edematous. The abdomen is greatly distended. Slight clubbing and cyanosis of digits is observed.

On opening the abdomen the uterus projects from the pelvis to the level of the umbilicus and contains a ballotable fetus.

The chest contains scanty thymic remains, the pleural cavities are dry and the right lung is bound down posteriorly by dense, fibrous adhesions.

Heart: The pericardium contains about 50 cc. of yellow fluid. The heart weighs 375 gm. The wall of the left ventricle appears slightly hypertrophied but the chambers appear normal. Along the line of closure of the aortic leaflet of the mitral valve is a mass of friable vegetations measuring 2 by 1.5 by 1 cm. that are dark red, granular and crumbly in consistence. There are no extensions along the chordae tendineae to the papillary muscles or to the auricular endocardium. The line of closure of the posterior mitral leaflet is thickened and contains some minute vegetations. Some of the chordae tendineae are slightly thickened and shortened. No old or recent aortic, tricuspid or pulmonary valvulitis is present. Coronary orifices are patent. Vessels show no sclerosis. Myocardium is dark reddish brown. Over the anterior surface of the left ventricle near the apex, directly along the course of the anterior descending branch of the coronary artery, is a circumscribed area of pallor and softening without thinning of the wall. There is a similar area over the posterior surface of the left ventricle near the apex along the course of the circumflex branch. These are recent infarctions.

Lungs: The lungs are smooth save for dense fibrous adhesions over the right lower lobe. The bronchi contain a slight amount of

mucopus. Both lungs contain diffusely scattered, subpleural, dark red areas of infarction, presenting a contrast to the pink lung. These are even more noticeable in the parenchyma.

Spleen: The spleen weighs 800 gm. and measures 15 by 9 by 5 cm. Its surface is slate gray with contrasting yellow areas of infarction varying from 1 to 3 cm. in diameter. On section pulp is soft and bright red, with ragged subcapsular infarcts occupying about 50 per cent of the organ.

Liver: The liver is large, yellow and fatty, and weighs 3000 gm.

Kidneys: The kidneys are slightly enlarged. Capsules strip readily exposing a smooth purplish red surface interrupted by depressed, indented, irregular infarcts varying from 1 to 2 cm. in diameter. Cut surfaces are bluish red in color with infarcts cutting deeply into the organs. The kidney substance does not bulge beyond the capsule. The markings are obscured. Pelves appear congested. The glomeruli are not enlarged. No petechiae are present.

Uterus: The uterus contains a well formed fetus 26 cm. in length. Its placenta is intact.

Iliac Arteries: Distal to the bifurcation of the aorta both common iliac arteries are thrombosed as follows: the proximal 2 cm. of the right common iliac is uninvolved; the distal portion contains a firm, yellow-gray thrombus about 2.5 cm. long extending for about 0.5 cm. into the corresponding hypogastric artery. On the left side the clot begins about 0.5 cm. below the bifurcation of the aorta and is about 4 cm. long. The proximal 3 cm. is dark red, the distal cm. yellow and firm, extending for about 0.5 cm. into the left hypogastric artery.

Microscopic Examination

Tissues for microscopic sections were removed from the various organs and fixed in 10 per cent formol and in Zenker's fluid and then embedded in paraffin. Heart sections were removed after the routine manner suggested by Gross and coworkers.[6] These were stained with hematoxylin and eosin, by Giemsa's method, and by Brown and Brenn's picric acid differential method.[7]

Heart: Gram stains of smears from the vegetations reveal large numbers of Gram-negative bacilli arranged in clusters and short

chains. These organisms exhibit bipolar staining and vary considerably in size and length.

The anterior leaflet of the mitral valve is largely replaced by a mass of vegetations in the base of which a remnant of old valve substance can be recognized. This remnant shows no vascularization or evidence of antecedent rheumatic change. The vegetation consists of a crumbly mass of fibrin and leukocytes in the midst of which Giemsa sections disclose the presence of enormous numbers of Gram-negative bacilli. Many are intracellular both in polymorphonuclear leukocytes and in underlying connective tissue macrophages. The deeper zone of the vegetation is composed of cellular granulation tissue.

The posterior mitral leaflet is covered with several minute verrucae that consist of small subendothelial elevations covered with fibrin. The bases of these elevations consist of closely packed fibroblasts. No microörganisms can be found in this leaflet. Between the small verrucae the valve surface is covered with a column of cells forming a palisade usually 2 or 3 rows in thickness. These cells are directed obliquely or at right angles to the valve surface, and are often covered with a thin layer of fibrin. The cells are elongated with large basal nuclei. Within the valve substance appear foci of fibrinoid necrosis. The subendothelial connective tissue is swollen and hyalinized and surrounding these foci are accumulations of round cells. Large numbers of basophilic mast cells lie beneath the endocardium of the left ventricle, which like the valvular endothelium, is often replaced by a cellular palisade. Mast cells also appear throughout the interstitial tissue of the myocardium where, together with polymorphonuclear and mononuclear leukocytes, they surround coronary venules. The vascular endothelium is flattened and beneath it is a row of clearly staining cylindrical cells directed at right angles to the endothelium.

The muscle fibers in the mitral ring are hyalinized. The endothelium of the left auricle appears normal, but the subintimal tissue is infiltrated with fibroblasts, polymorphonuclear cells and histiocytes. No Aschoff bodies are found in the auricular myocardium.

Purulent emboli are seen in the small branches of the left anterior and posterior descending coronary arteries and in small branches to the papillary muscles. These consist of fibrin clumps with necrotic polymorphonuclear cells, but in places contain proliferating fibro-

blasts. The media is destroyed at one side with resultant formation of a mycotic aneurysm. This purulent process undergoes extension into the adjacent myocardium where extensive infarction, muscle degeneration, polymorphonuclear infiltration and scars of granulation tissue are found. Giemsa sections reveal no microörganisms in the infarcted areas.

Lungs: Marantic infarcts occupy large areas of the sections. The smaller bronchi and terminal bronchioles contain plugs of mucopus. The bronchial walls are infiltrated with lymphocytes and plasma cells. Surrounding them are well circumscribed islands of connective tissue densely infiltrated with round cells and containing "glandular structures" lined by cuboidal epithelium.

Liver: The liver sinusoids are packed with every variety of bone marrow cell including all types of granular leukocytes, together with megakaryocytes and large mononuclear cells. The lymphoid elements in the portal spaces are hyperplastic and contain many myeloid cells. There is an astonishing number of immature forms, myelocytes, band forms and metamyelocytes of the neutrophilic and eosinophilic series. These are found in the portal veins where they appear to marginate and invade surrounding sinusoids.

Spleen: The spleen contains wedge-shaped peripheral infarcts. In the splenic arterioles there are granular leukocytes of varying stages of maturity. The lymphoid follicles are hyperplastic. Many leukocytic cells and megakaryocytes of the same type as were present in the liver lie beside the endothelial sinuses of the pulp.

Kidneys: The majority of the glomeruli are intact but in many of them focal embolic lesions are identified by necrosis of one or more tufts. In some instances emboli lie within afferent glomerular arterioles. Some emboli contain bacteria morphologically identical with *Hemophilus hemolyticus*. The capsules of the affected glomeruli are surrounded by zones of polymorphonuclear leukocytes and round cells. A few epithelial crescents are present in Bowman's capsule. There is a moderate degree of tubular degeneration with exudation of albumin into the lumen and occasionally hemorrhage. The vessels appear intact.

Aorta: In sections through each common iliac artery the middle coat is invaded by polymorphonuclear leukocytes separating the smooth muscle fibers. Both the cells and the medial coat are under-

going necrosis. The vasa vasorum contain numerous polymorphonuclear leukocytes in process of margination. Overlying the intima is a huge thrombus which contains no bacteria.

DISCUSSION

So far as can be determined this is the second case of bacterial endocarditis to be reported in which *Hemophilus hemolyticus* has been the cause. There are several features in this case that call for comment and it is proposed to discuss them in the following order: (1) the bacteriology indicating a mixed infection with *Hemophilus hemolyticus* and *Streptococcus viridans*; (2) the association with pregnancy; (3) the probable primary focus; (4) the nature and significance of a myeloid reaction in the liver and spleen; (5) the unusual embolic phenomena; and (6) the relation of the verrucous endocarditis on the posterior mitral leaflet to the vegetative endocarditis on the anterior mitral leaflet.

There can be little doubt that in this case death was due to infection by *Hemophilus hemolyticus*, probably associated with *Streptococcus viridans*. Proof of infection by the former organism consists in the recovery in blood culture, its presence in smears and in the substance of the vegetation, and finally in its presence within the embolic glomerular lesions. Because the valvular and embolic glomerular lesions were partially productive in character and hence of some chronicity, it would seem reasonable to assume that *Hemophilus hemolyticus* was the primary infective agent. Moreover, in terminal invasions associated with endocarditis it is uncommon to find the infective microörganisms deep in the substance of the vegetations, as in this case, although they may occur on the surface.

The rôle of *Streptococcus viridans* is difficult to interpret. Although the organism is the most frequent single causative agent of bacterial endocarditis, its absence from the heart valve *in the presence of a bacteremia* is unusual. In twelve cases of bacterial endocarditis in which blood culture yielded non-hemolytic streptococci Wright [8] was able to demonstrate the microörganisms in the valve section in eleven instances. It would seem, therefore, that this organism is to be found in the valve substance in a high percentage of cases where it is the responsible agent and when blood culture is positive. The frequency with which *Streptococcus viridans* is a secondary invader in rheu-

matic and other infections, together with its absence in the histological preparations, are strong arguments in favor of a secondary infection.

Pregnancy as a complication of bacterial endocarditis is of some interest. The literature mentions relatively few cases with this feature. Kobacker [9] recently reported a case and quoted Walser who, in an extensive review of the literature, was able to find but two bona fide cases of endocarditis lenta complicated by pregnancy. Walser added two cases of his own. Blumer's comprehensive monograph [10] fails to mention the subject, from which it may be deduced that such an association is infrequent. The fetus in our case was well formed and corresponded to that of a 6 or 7 months pregnancy.

The organizing bronchopneumonia suggests itself as a probable portal of entry. The connective tissue reaction in and about the bronchial wall indicated a process of some chronicity. Blumer's analysis mentions the bronchi as a probable focus of infection in five out of fifty-eight cases of bacterial endocarditis.

The myeloid reaction present in the liver and spleen, as revealed by Giemsa's stain, was so striking as to border on the changes of a myelogenous leukemia. *A priori* there are two sources from which bone marrow cells in extramedullary sites may be derived: (1) from preëxisting cells present in those organs by a process of so-called extramedullary myelopoiesis, and (2) as circulating deposits from the bone marrow.

Maximow [11] has demonstrated the formation of bone marrow within the kidney of the rabbit after ligation of the renal arteries and vein. He believed that the endothelial cells of the liver, along with the fixed tissue cells or histiocytes, were endowed with unrestricted mesenchymal potentialities, among which was the ability to form hemocytoblasts from which all successive varieties of bone marrow cells could be derived. Whenever extramedullary myelopoiesis occurred myelocytes appeared first, followed in order by megakaryocytes and erythroblasts, but often the erythroblast stage was not reached.

Evans [12] studied the splenic tumors resulting from infections and distinguished a gray and a red variety. The former occurred in bacterial endocarditis and was characterized microscopically by the presence of numerous myeloid forms. These, he believed, arose from mononuclear myeloid cells normally present in the pulp, but indis-

tinguishable by ordinary staining methods. The red variety was typified by the splenomegaly of typhoid fever in which the predominant cell was an endothelial or mononuclear cell.

Histological examination will not absolutely solve the problem of the origin of these extramedullary myeloid cells. We feel, however, that origin from circulatory deposits is the more likely because the myeloid cells could be traced to the splenic arterioles and to the portal veins. The presence of the young myeloid forms indicates a severe and overwhelming demand upon the bone marrow for leukocytic cells which can be met only by discharge of immature forms.

The embolic phenomena here call for some consideration. Embolic deposits in the left coronary artery with accompanying focal purulent myocarditis were present and appeared identical with lesions reported by Miller and Branch, and by Russell and Fildes. These emboli were probably derived directly from the anterior mitral vegetation. Histologically they were identical with the superficial layers of the vegetations. The infrequency of such emboli may be inferred from the failure of Blumer to mention them in his monograph.

The embolic phenomena in the kidneys are the classical focal Löhlein and Baehr [13] lesions with *Hemophilus hemolyticus* demonstrable in glomerular tufts. The splenic and renal infarcts are typical. The thrombi in the iliac arteries are localized over areas of purulent destruction of the media, probably from small emboli in the vasa vasorum.

Finally it is interesting to contrast the reactions on the anterior and posterior mitral leaflets. The former was essentially an exudative reaction to bacteria, the latter essentially proliferative and bacteria-free. In fact, the gross and the histological appearance of the vegetations on the posterior leaflet was similar to that seen in acute rheumatic valvulitis, a similarity that has previously been recognized by Clawson and Bell.[14] Recently Leary [15] has described a proliferative reaction of the valve surface in rheumatic valvulitis which he has termed a "palisade formation" and which he regards as the earliest valvular response to the action of bacteria. VonGlahn,[16] Swift [17] and others have called attention to focal degeneration of collagen and elastic tissue of the rheumatic valve surrounded by proliferating cells as a pathognomonic feature of rheumatic inflammation and a precursor perhaps to the Aschoff body. Histologically, it will be recalled, the posterior mitral leaflet showed a type of

reaction similar to, if not identical with, the palisade reaction, as well as a fairly extensive area of fibrinoid swelling and degeneration within the substance of the valve surrounded by cellular reaction. The valvular responses of bacterial endocarditis may thus occasionally imitate those of rheumatic endocarditis.

Summary

1. A case of *Hemophilus hemolyticus* endocarditis associated with *Streptococcus viridans* infection is reported. It represents the second recorded instance of infection by the former microörganism.

2. The infection was complicated by pregnancy.

3. A probable portal of entry is demonstrable in the form of an organizing bronchopneumonia.

4. A myeloid reaction is exhibited in the liver and spleen and probably represents the effect of a severe and prolonged over-stimulation of the bone marrow by a bacterial toxin, with a resultant deposition of many immature marrow cells in the hemopoietic organs.

5. Some unusual embolic phenomena are present in the coronary arteries.

6. A villous endocarditis on the anterior mitral leaflet abounding in bacteria is contrasted with a proliferative reaction on the posterior leaflet. This latter reaction, together with a type of recently described "palisade formation," is here shown for the first time in subacute bacterial endocarditis. The existence of an additional lesion, described as a fibrinoid or hyaline swelling and degeneration of collagen valve substance previously demonstrated in rheumatic valvulitis and in rheumatic auricular endocarditis is shown in subacute bacterial endocarditis.

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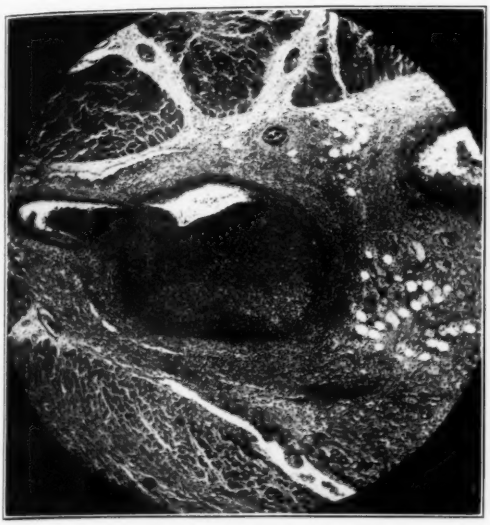
DESCRIPTION OF PLATE

PLATE 62

FIG. 1. A branch of the left anterior coronary artery with an embolic occlusion is seen. The surrounding myocardium is infarcted. Hematoxylin-eosin stain. × 70.

FIG. 2. Myeloid reaction in liver. Many immature leukocytes may be seen in a portal space and among the sinusoids. Giemsa stain. × 150.

FIG. 3. Focal embolic glomerulonephritis. Note the bacteria within the afferent arterioles and the surrounding cellular reaction. Hematoxylin-eosin stain. × 250.



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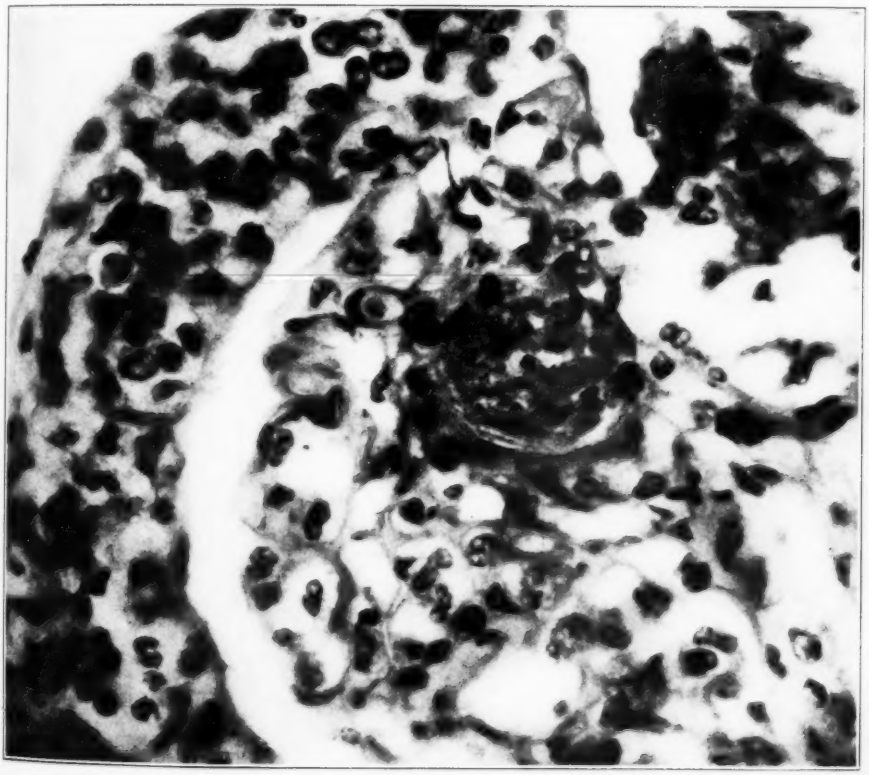

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De Santo and White Hemophilus Hemolyticus Endocarditis